BILATERAL HEREDITARY MICRO-EPIPHYSEAL DYSPLASIA

Clinical and genetic analysis of a Dutch family

Bilaterale hereditaire micro-epifysaire dysplasie

Klinisch en genetisch onderzoek van een Nederlandse familie

(met een samenvatting in het Nederlands)

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Ter verkrijging van de graad van doctor aan de Universiteit Utrecht
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Copromotores: Dr. B.R.H. Jansen, orthopaedisch chirurg
Prof. Dr. P. Heutink, moleculair geneticus

Like apples of gold in settings of silver,
so is a word spoken at the right moment.

Proverbs 25:11

To Cora, Jan-Hans, Pauline and Martijn
My father, mother and brother

CONTENTS

CHAPTER 1

INTRODUCTION

Congenital anomalies and genetic diseases are the most important single causes of disease and mortality in the first year of life in Western countries. There are, at present, more than 500 disorders known in which the skeleton is involved. These are mostly congenital disorders and are, in part, genetic in nature. The frequency of each disorder is low but, as a whole, they constitute a significant body of disease in childhood. These disorders lead to important diagnostic, clinical, genetic and therapeutic challenges. Reliable estimates of its frequencies are scarce.

The complexity of skeletal disorders, the many types, the heterogeneity within each type and the non-uniform terminology used in the international literature caused a great deal of confusion in the classification in the past. Continuing efforts over the years by a number of experts have helped to clarify well-known entities and have delineated newer disorders. This resulted in the International Nomenclature of Constitutional Diseases of Bone, which was issued in 1969 under the auspices of the European Society of Paediatric Radiology (Maroteaux, 1970), and subsequently revised in 1977, in 1983 and 1997 (Rimoin, 1998). The last revision of the International Classification of Constitutional Disorders of Bone was published recently (Hall, 2002). An on-line version is available on http://www.csmc.edu/genetics/skeldys/ (see Appendix 2). The International Nomenclature is based on the clinical, genetic and radiographic characteristics of each condition. Additional delineation of disorders was done whenever specific morphological or biochemical defects had been demonstrated. The arrangement and subdivisions for nomenclature do not constitute a real classification and their only purpose is to clarify the terminology and facilitate further research. The terminology is rather descriptive, covering the aspect of the skeleton, the part of the skeleton that is affected in radiographs or sometimes the consequences of the abnormality.

By definition, dysplasia is the process and the consequence of dyshistiogenesis, which exists in cartilage and/or bone of the whole skeleton. Dysostosis, on the other hand, is a disorder of individual bones as a single unit or in combination (Brons and van der Harten, 1988).

The three major physiologic functions of bone cells are calcium transport, biophysical or bioelectrical activities and hormonal responses. Normally, osteoblasts form bone, osteocytes contribute to mineral matrix homeostasis and osteoclasts resorb bone. Intrinsic or extrinsic factors capable of blocking or altering these major physiologic functions could have far-reaching effects on developing skeletal and connective tissue.

Many connective tissue disorders are believed to be caused by an abnormality in collagen, the major structural protein of connective tissue. The system for collagen production is complex, thus incurring the possibility of errors in the raw materials, in the enzymes required for assembly, and in the assembly process itself.

Constitutional diseases of bone with a known pathogenesis include chromosomal aberrations, metabolic abnormalities and especially also the mucopoly-saccharidoses. Those with an unknown pathogenesis include abnormalities of cartilage or bone growth and development, disorganised development of cartilage and fibrous components of the skeleton, malformations of individual bones, singly or in combination and abnormalities of density of cortical diaphyseal structure, of metaphyseal modelling, or of both. Bone abnormalities may also arise secondary to a disturbance in an extraskeletal system such as the endocrine, haematological, neurological, neuromuscular, renal, gastrointestinal or cardiopulmonary system.

In this thesis we will introduce another dysplasia to the list, called bilateral hereditary micro-epiphyseal dysplasia (BHMED). Since BHMED has a number of

features in common with multiple epiphyseal dysplasia (MED) as a broad and heterogeneous group of skeletal disorders, first the literature on MED and MED-like disorders is reviewed.

Even MED is a rare disorder. Its prevalence is estimated as 11.2 per 1 million based on index patients, and 16.3 per million, if affected relatives are included (Wynne-Davies, 1985). More recently, the prevalence has been estimated as 9:100,000 (Taybi and Lachman, 1996). In the Netherlands, one may expect about 168 to 244 patients with MED: every Dutch hospital cares for one or two patients with MED on average. At the Reinier de Graaf Hospital in Delft (the Netherlands), about 20 family members were known to have complaints with especially their hips and knees, suspected to have a variant of MED, BHMED as formerly proposed by Elsbach (1959). This was the starting point of a family study on the phenotypic spectrum of BHMED

Elsbach described this dysplasia in 1959 (Elsbach, 1959). As a Dutch orthopaedic surgeon, he examined family members on his outpatients' department, all with the same sort of complaints. These complaints started in the early childhood, bilaterally, symmetrically and synchronically. Because of the radiological finding of small epiphyses of the hip joint, he called his finding *bilateral hereditary micro-epiphyseal dysplasia (BHMED)*. Shortly after publication he retired and until now there has been no follow-up study on this family and its disorder.

Forty years later, we still have contact with the same family. Several members of the original family were referred to the department of orthopaedic surgery in Delft with questions about the genetic background, and the recurrence risk. We extended the pedigree and re-examined the extended family, in order to evaluate diagnostic criteria and the clinical course, to determine the clinical spectrum, and to

provide tools for the diagnosis of possibly affected persons with only partial expression of the disease.

During our investigations many members of the family told a remarkeble story about their common grandfather, who in the midst of the 19th century, on a journey through the Dutch Indies, was supposed to have been bitten by a crocodile. As far as they remember, the disease appeared in their family from that moment on!

REFERENCES

Brons JTJ, Harten van der JJ. Skeletal Dysplasias, pre- and postnatal identification. Thesis, 1988, Free University, Amsterdam.

Elsbach L. Bilateral Hereditary Micro-Epiphyseal Dysplasia of the hips. J Bone Joint Surg 1959;41B(3):514–523.

Hall CM. International Nosology and classification of constitutional disorders of bone (2001). Am J Med Genet 2002;113(1):65–77.

Maroteaux P. Nomenclature internationale des maladies osseuse constitutionelles. Ann Radiol 1970;13:455–464.

Rimoin DL. International Nomenclature and Classification of the Osteochondro-dysplasias (1997). Am J Med Genet 1998;79:376–382.

Taybi H, Lachman RS. Radiology of Syndroms Metabolic Disorders, and Skeletal Dysplasies, 4th Edn. Mosby, New York , 1996.

Wynne-Davies R, Gormley J. The prevalence of skeletal dysplasias. An estimate of their minimum frequency and the number of patients requiring orthopaedic care. J Bone Joint Surg 1985;67B:133–137.

CHAPTER 2

AIM OF THIS STUDY

In this thesis we will try to give a precise description of the phenotype as far as possible in a family with a wide spectrum of complaints and give an answer to the following questions:

1. Is BHMED indeed a separate clinical entity among the MEDs?
2. What are the diagnostic radiographic features of BHMED?
3. What is the molecular genetic basis of BHMED?
4. What is the diagnostic value of metacarpophalangeal pattern (MCPP) profile analysis?

CHAPTER 3

REVIEW OF THE LITERATURE OF MED AND MED-RELATED DISORDERS

3.1. CLINICAL SYMPTOMS

In this chapter, the different clinical features of the multiple epiphyseal dysplasias (MEDs) are reviewed. There are more than 100 disorders of cartilage development, many of which are most likely due to genetic defects in the macromolecular components of the growing or resting cartilage. A comprehensive approach dealing with all forms of the disease is hampered by extensive clinical overlap and undetermined levels of genetic heterogeneity. In this study, we will focus on the clinical phenotypes of MED and a number of related disorders with currently known genetic defects.

MED is one of the most common osteochondrodysplasias (Wynne-Davies and Gormley, 1985) and the most common disorder in the group of generalized epiphyseal disorders with predominantly limb involvement (Spranger, 1976). This dysplasia was well described by Fairbanks in 1935. It is an ossification disturbance of the epiphyses of the long bones, which leads to a serious deformation of the involved joint. When the vertebrae are also affected, the term spondyloepiphyseal dysplasia (SED) is used. Most forms of MED have an autosomal dominant pattern of inheritance with a high penetrance and variable expression (Tachdjian, 1990). In addition, at least one autosomal recessive form is known. The disease gives rise to early-onset osteoarthritis of the major joints of especially of the lower limb, but also of the upper limbs (van Mourik, 1993). Due to growth retardation of the epiphyses of the tubular bones, short and stubby finger ends are seen. The contour of the epiphyses is roentgenologically irregular and fragmentized with an inhomogeneous osseous structure. The ossification centres appear at different times, which gives the impression of stippled epiphyses which is due to the multiple calcifications in or near the epiphyses.

Originally, it was thought that shortness of stature and brachydactyly constituted the basic features of MED, but subsequent studies recorded persons of normal height and without digital abnormalities (Hoefnagel et al., 1967). In fact, extensive clinical variability even within one family was observed, which suggests that in addition to the major gene defect other factors determine the expression of the disease. Maroteaux (1969) has defined MED as "a group of conditions in which the changes in the long bones are limited to the epiphyses and the spinal lesions are limited to the vertebral plates without notably affecting the height of the vertebrae". Today, this is probably the best definition available, although the clinical boundaries of the different epiphyseal dysplasias are not easy to interpret.

With the possible exception of the pseudoachondroplasias, skeletal abnormalities of the MED-type are not visible at birth. In fact, significant manifestations are usually not seen until mid-childhood (ages six or seven). When they do appear, the disturbances of the skeleton are likely to be bilateral and symmetrical and, in contrast to those of the osteochondroses (Breck, 1971), involve three or more joints. During childhood and adolescence it affects the epiphyses of the tubular bones, resulting in axial deformities and sometimes in shorter limbs. Later in life, MED may lead to early-onset osteoarthritis. Possibly, the non-specific label of 'bilateral premature osteoarthritis', may sometimes in fact hide underlying skeletal dysplasias like MED. Up until now, the diagnosis of MED was solely based on physical examination and radiological survey. The presenting features of MED include stiffness in the affected joints, development of pain, a limp or waddling gait, difficulty in climbing stairs and running (Rimoin and Lachman, 1993; Dee, 1997). There is a limited range of motion in the affected weight bearing joints. It is usually noted in childhood, sometimes in infancy, and occasionally only incidentally in adult life. There is mildly short stature, with an adult height of rarely 145 cm and more usually around 160 cm, and short, broad hands and thumbs.

Valgus deformities of the knees and ankles are common. In childhood, a deficiency of the lateral part of the distal tibial ossification centre is seen, and this may develop towards a sloping end of the tibia in adulthood. The diagnosis in the adult is aided by the changes in the distal tibia. Facial appearance and intelligence are normal (Leeds, 1960).

Radiographic abnormalities of the skeleton appear after the third year of life whereas there are usually none at birth (Beals and Horton, 1995). The most important finding is the delay and irregularity of ossification of the involved epiphyses. Radiographic appearance includes delayed appearance of the secondary centres of ossification, including the proximal femoral ossification centre. There is often a patchy, irregular ossification of the involved epiphyses, which is frequently symmetrical on both sides and more pronounced in the lower extremities. In severe cases, the adjacent metaphysis is also splayed and irregular. The limb epiphyses gradually enlarge and the irregular areas coalesce. The capital femoral epiphyses are nearly always involved and subsequently the femoral head is flattened, sometimes with poor coverage and subluxation, and sometimes deeply set with a protrusion of the acetabula. The acetabulum is occasionally normal, but more often there is some loss of definition with a scalloped outline. In some reported families no hip abnormalities were observed. There is a delay in the appearance of all secondary ossification centres of the tubular bones, including the hands and wrists. The epiphyses of all long bones can be affected and there may be some irregularity of the end plates in the lower dorsal spine, but in general this is minor. Based on the radiographic appearance of the pelvis and femoral heads, the disorder can be confused with bilateral Legg-Calvé-Perthes' disease, but the changes are symmetric, and involve the metaphyses rather than the epiphyses of the hip. Osteonecrosis may develop in addition to the underlying dysplasia, and it seems to follow the same pattern of development as in Legg-Calve-Perthes' disease. This

latter disorder is still poorly understood, in aetiology as well as in treatment, with controversies about all aspects. Although a metabolic basis for the disorder has been suggested, up to the present day none has been found. Legg-Calvé-Perthes' disease is bilateral in 15% of the cases, but it is rarely symmetric (Crossan et al., 1983). Moreover, in contrast to MED, Legg-Calvé-Perthes' disease is transient, with initially an active phase of deterioration followed by improvement and healing. MED shows only steady progression during growth (Crossan et al., 1983). Bilateral Legg-Calvé-Perthes' disease affects only the capital femoral epiphyses, although growth and bone maturation may be delayed. In Legg-Calvé-Perthes' disease there are patches of increased density in the capital epiphyses and often clear metaphyseal involvement with cyst-like changes. The acetabulum is normal or only very slightly affected (secondarily) by changes in the shape of the developing femoral head. Generally, once re-ossification has started the potential for further femoral head deformity is minimal, although new information suggests that some hips become progressively flattened after onset of re-ossification. This phenomenon is more likely to occur in older patients, in those with more severe lateral pillar classification, and in those who have a prolonged re-ossification phase.

MED can also but less frequently be confused with Meyer's dysplasia, although in this dysplasia, only the femoral head is affected on both sides. However, MRI studies have shown that most patients actually have changes compatible with superimposed avascular necrosis. The natural history of hip development in these patients shows that all incongruent hips are osteoarthritic by the age of 20 (Meyer, 1964; Treble et al., 1990; Dee, 1997).

Patients with minor epiphyseal abnormalities of the shoulder have been shown to develop painful osteoarthritis in middle age, but they retain shoulder movement

until the degenerative changes are advanced. Those with a significantly deformed 'hatchet head', radiographic appearance of the humeral head were shown to have had minimal glenohumeral movement from an early stage onwards, and this condition often became painful by the fifth and sixth decades (Ingram, 1991). Epiphyses may arise from more than one centre of ossification. The accessory centres of ossification in MED are few in number and scattered at the periphery of the cartilaginous epiphysis.

In MED, the metacarpals and phalanges are usually short and the finger ends are stubby. The 'V' wrist-joint deformity in MED is due to hypoplasia of the adjacent portions of the radial and ulnar epiphyses (Hoefnagel et al., 1967). The carpal bones are small and irregular in MED.

The 'slant sign' of the ankle (thinning of the lateral part of the lower tibial epiphyses), supposedly a characteristic feature of MED, is rather non-specific. A 'flat-top' talus is also seen in some MED subtypes. Flattening of the intercondylar notch of the knee is a helpful early finding, but not specific for MED. Later, the femoral condyles often show irregular ossification. Femoral head changes are variable: they may be absent, grossly deformed, irregularly ossified (as in Legg-Calvé-Perthes' disease), or normal (Barrie et al., 1958). Progressive flattening and enlargement of the femoral head is characteristic in MED and eventually leads to secondary osteoarthritis.

In MED, the vertebral bodies are of normal height but may show end-plate irregularity. In the spine, there is not a real flattening or reduction of the vertebral bodies, but the observed vertebral changes may be quite similar to Scheuermann's disease due to the intraspongious herniations of the lower thoracic or first lumbar vertebrae. With the appearance of the apophyses, the rim of the vertebral body shows fragmentation and, infrequently, a vertebral body will be fragmented (Rubin, 1964).

Radiographic anthropometric studies may be of some value in the early diagnosis of MED (Poznanski et al., 1972; Ingram, 1992). Typical epiphyseal changes may be present in the joints of apparently healthy family members. These differences may depend upon the age, height, weight, and activity of the patient (Silverman, 1968).

Clinically, MED was separated into two major forms of the disease, a severe form known as the Fairbanks type, and a milder form, in which mainly the hips are involved, known as the Ribbing type.

Clinical variability versus genetic heterogeneity is a major issue in MED and related disorders. Forms of MED more severe than those reported by Fairbanks or less severe than those reported by Ribbing have been described (Odman, 1959; Cameron and Gardiner, 1963; Hulvey and Keats, 1969; Diamond, 1970). A number of families have been reported in which MED was the presumptive diagnosis, but with additional features such as spinal abnormalities suggestive of SED and early-onset osteoarthritis of the major joints (Cameron and Gardiner, 1963), variable spinal involvement (Hulvey and Keats, 1969), which led to attempts to distinguish subforms on the basis of spinal abnormalities (Diamond, 1970). Marked hyperextensibility of the fingers, early-onset osteoarthritis of the hips and short stature were reported by Bachman and Norman (1967).

Villareal et al. (1992) wrote about a family with short hands and feet and, radiographically, a short fourth metatarsal bone.

None of the known MED types involves the scapula, sternum, pelvis, ribs, clavicle, mandible, skull, face, or teeth.

Stanescu et al. (1993) described similarities between the upper tibial cartilage of MED-Fairbanks-type and pseudoachondroplasia (PSACH). They suggested that both are allelic disorders.

Pseudoachondroplasia (PSACH), formerly called pseudoachondroplastic dysplasia, is not easy to differentiate from other severe forms of MED; but in the former there is severe disproportionate dwarfing with very short limbs and marked metaphyseal involvement. Some patients have striking joint laxity, a feature seldom present in MED, but possibly present in the family described by Bachman and Norman (1967).

PSACH is one of the most frequent skeletal dysplasias. The patients appear normal at birth and growth retardation is seldom recognized until the second year of life or later, at which time the body proportions resemble those of persons with achondroplasia. The facies of patients with achondroplasia are similar and normally shaped. The fingers are short and do not show the trident configuration typical of achondroplasia. They are hyperlax and can be pulled out in a telescoping fashion. Deformities of the lower limbs range from genu varum to genu valgum to 'wind-swept' deformity. Ligamentous laxity contributes to the leg deformities. There is incomplete extension at the elbows and ulnar deviation of the wrists. The child has a relatively long trunk, short extremities and increased lumbar lordosis. The diagnosis of PSACH is easily made from pelvic radiographs. The development of the whole pelvis is much delayed and disorganized, with poor formation of the acetabulum and widening of the triradiate cartilage and epiphyseal and metaphyseal changes most notable at the proximal and distal femoral ossification centres (Apley and Solomon, 1993). Radiologically, all tubular bones are short with widened metaphyses and fragmentation and irregularities of the developing epiphyses. The epiphyses of the hips and phalanges are small, i.e., 'mini epiphyses'. In childhood, platyspondyly is characteristic, with anterior tonguing due to delayed ossification of the annular epiphyses (Rimoin et al., 1994).

Langer et al. (1993) presented a 7½-year-old girl with achondroplasia ánd PSACH. Her mother had achondroplasia and her father had PSACH. Langer et al.

(1993) outlined the radiographic manifestations of these conditions and compared the findings in this patient to those of achondroplastic and pseudoachondroplastic patients of similar ages. Their review of these radiographs and of those of patients with the Fairbanks type of MED led them to the conclusion that Fairbanks-MED may be the mildest form of PSACH, a conclusion also suggested by the linkage studies mentioned further on.

Stanescu et al. (1982) found an accumulation of a non-collagenous protein in the rough endoplasmic reticulum of chondrocytes and the absence of a proteoglycan 'population' from cartilage. The authors suggested that an abnormally synthesized or processed protein core is not properly transferred to the Golgi system.

To delineate the natural history of PSACH at all ages, Mc Keand et al. (1996) collected questionnaire information on 79 affected individuals. The phenotype was not distinct or more severe in familial cases as compared with new mutation cases. Furthermore, there were no differences in the number of orthopaedic complications, operations or number of offspring between these 2 groups. Less than half the affected adults reported having a total hip replacement surgery. Extraskeletal complications were generally uncommon. Premature osteoarthritis was the major health problem.

SED versus MED

In view of spinal involvement in some families diagnosed with MED, the question was raised whether MED and SED belong to a single major category of skeletal dysplasias. In view of the description of one of his families, Maroteaux (1969) seemed willing to accept a slight degree of platyspondyly in MED.

On the other hand, SED may also show a large variety in the severity of spinal abnormalities, and a wide range of additional clinical features is reported such as

short trunk, barrel chest, increased thoracic kyphosis, marked lumbar lordosis, and short limbs with relatively normal hands and feet, genu varum or valgum, muscular hypotonia, waddling gait, and early mild degenerative changes of the hips. The radiographic appearance of the skeletal system includes delayed ossification of the pubic bone and the proximal and distal femoral epiphyses, pear-shaped vertebral bodies in infancy, flattening and irregularity of ossification centres in childhood, and severe vertebral flattening by adulthood. Dysplasia of the femoral head with progressive degeneration is present. Hypoplasia of the odontoid process may lead to cervical instability with spinal-cord compression. Kopits et al. (1974) described a 12-year-old patient with chronic compression myelopathy of the cervical cord due to habitual atlantoaxial dislocation. The usual presentation is generally one of slowly progressive loss of motor strength.

A subtype of SED, spondyloepiphyseal dysplasia tarda (SEDT), is usually diagnosed in adolescence and is considered to be an X-linked recessive condition (Tachdjian, 1990; Ikegawa, 1993; Ikegawa et al., 1993). Clinical manifestations include short stature, mainly short trunk, dorsal kyphosis and lumbar hyperlordosis, and back and hip pain associated with progressive limitation of joint range of motion, mainly hip flexion contractures. Radiographic appearance includes platyspondyly with a hump-shaped deformity in the posterosuperior aspect of the vertebral bodies and narrowed disc spaces seen on the lateral view. Ossification of the upper and lower anterior margins of the vertebral bodies is absent (Wynne-Davies et al., 1982; Wynne-Davies and Hall, 1982).

Mild epiphyseal dysplasia with short femoral neck and coxa vara can be seen with early degenerative changes of the hips; distal epiphyses of the long bones and hands and feet are not affected.

Concerning the differential diagnosis of the different epiphyseal dysplasias, it is a problem to define clear-cut boundaries between the different phenotypes. Rarely

two families with the same disorder or two members of the same family show similar patterns of involvement. Furthermore, one or two members within an affected family with the diagnosis of a MED-like disease may demonstrate findings suggestive of another condition, e.g., SED or PSACH. In addition, the clinical variability does not only manifest itself in the autosomal dominant forms, but may also be present in families with apparently autosomal recessive patterns of inheritance.

3.2. GENETIC ASPECTS

Over 100 unique skeletal dysplasia phenotypes have been clinically defined (International Working Group on Constitutional Diseases of Bone, Hall, 2002; http://www.csmc.edu/genetics/skeldys/). The primary features that separate one phenotype from another are clinical presentation and natural history, radiographic abnormalities, inheritance pattern and morphology of cartilage or bone. In a few of these disorders, the primary genetic defects have been identified, but the majority are aetiologically undefined.

Spranger (1985) has proposed classifying clinically similar phenotypes that likely share a common pathogenetic mechanism into bone dysplasia families while recognizing the great diversity in phenotypes associated with genetic defects in a specific pathway. Indeed, allelic disorders may span a spectrum of clinical severity depending on the nature of the mutation in the gene or genes that encode the molecule of interest. This concept fits the known variability of osteogenesis imperfecta phenotypes, all of which can result from dominant mutations in type I procollagen genes (Byers, 1990). Similarly, type II procollagen gene mutations have been characterized in individuals with phenotypes that range from the

perinatal lethal achondrogenesis type II phenotype to Stickler syndrome (arthro-ophthalmopathy) which is well compatible with adult life (Lee et al., 1989; Vissing et al., 1989; Ala-Kokko et al., 1990; Tiller et al., 1990; Ahmad et al., 1991; Ahmad et al., 1993; Chan and Cole, 1991; Horton et al., 1992; Bogaert et al., 1992; Brown et al., 1992; Winterpacht et al., 1993; Vikkula et al., 1993).

Similarly, clinical, morphological and histological features suggested that PSACH and the Fairbanks type of MED belong to the same distinct bone dysplasia family (Stanescu et al., 1993; Rimoin et al., 1994).

Though most MED conditions appear to have autosomal dominant inheritance, autosomal recessive types have also been reported, making them one of the first indicators of genetic locus heterogeneneity and different kinds of pathway involved. Juberg and Holt (1968) tabulated 11 reports in which more than one member of a sibship was affected while the parents were normal and often related. These cases included those of Barrington-Ward (1972), Ribbing (1937), Fairbanks (1947), Lodes (1949), Emr (1952), Watt (1952), Waugh (1952), Litchman and Chirls (1958) and families 28, 31 and 37 of Hoback (1961). Of course, one has to keep in mind the possibility that multiple affected siblings with unaffected parents may also represent cases of parental mosaics for an autosomal dominant gene defect, especially when the frequency of parental consanguinity is much lower than expected relative to the frequency of the disorder and the frequency of consanguinity in the general population studied. Secondly, the same gene may sometimes be involved in both AD and AR variants of the disease.

Based on thorough and careful clinical evaluation and radiological examination, different phenotypes within the MED spectrum can be distinguished. Indeed, genetic studies including linkage analysis, whole genomic searches and mutation analysis revealed at least five possible loci for MED, EDM1-5.

EDM1. The first gene for MED that was discovered was the COMP gene. By linkage studies, Oehlmann et al. (1993) mapped a locus for MED close to the centromere of chromosome 19. The highest LOD-score was observed for D19S199: maximum LOD = 4.67 at theta = 0.09. Subsequently, Oehlmann et al. (1994) reported a maximum LOD score of 6.37 at theta = 0.05 for linkage with D19S215. Multipoint linkage analysis indicated that MED was located between D19S212 and D19S215, a map interval of 1.7 cM. The gene for PSACH was mapped also to the pericentric region of chromosome 19. This finding, combined with the morphologic similarity between PSACH and MED suggested the possibility of allelism of the two disorders.

Deere et al. (1995) confirmed the linkage of autosomal dominant MED to D19S212, maximum LOD = 3.22 at theta = 0.00. However, in another family in which three of seven sibs were affected and the parents were unaffected, they excluded linkage to chromosome 19 (using both autosomal recessive and autosomal dominant models, with either reduced penetrance or germline mosaicism considered). Linkage to a number of other candidate genes, COL9A1, COL9A2 and COL11A2, was tested and excluded for both genetic models in the latter family.

Knowlton et al. (1995) further narrowed the possible location of the EDM1/PSACH gene to an interval of approximately 600 kb which included the COMP gene as a candidate gene. Subsequently, mutations in the COMP gene were found in both MED and PSACH (Hecht et al., 1995; Briggs et al., 1995), confirming that at least one form of MED and PSACH represent allelic disorders.

Table I summarizes the currently known allelic variants of COMP and associated phenotypes.

Table I. Allelic variants (selected examples) of cartilage oligomeric matrix protein (COMP) according to OMIM.

	Allelic variant	Author/year		Nucleotide	Result
PSACH	[COMP,ASP472TYR]	Hecht et al., 1995	G-to-T transversion	1439	Asp472-to-tyr Aa substitution
PSACH	[COMP,CYS468TYR]	Hecht et al., 1995	G-to-A transition	1428	Cys468-to-tyr Aa substitution
PSACH	[COMP,3-BP DEL, 459TCA, SER459DEL]	Hecht et al., 1995	deletion	1400-1402 TCA	Serine-459 deletion
PSACH	[COMP,3-BP DEL, ASP DEL, (GAC)4]	Hecht et al., 1995	deletion 3-bp	1430-1445 cDNA	Loss of aspartate residue in a calcium-binding site
PSACH	[COMP,3-BP DEL, ASP DEL, 9GAC)4]	Briggs et al., 1995	deletion 3-bp	1139-1147	Elimination of aspartic acid codon (372-374)
MED (Fairbanks)	[COMP,ASP342TYR]	Briggs et al., 1995	Tyr for asp substitution		RsaI restriction endonuclease cleavage site
PSACH	[COMP,CYS328ARG]	Briggs et al., 1995	Cys328-to-arg substitution		Conserved residue in second calmodulin-like repeat
MED (Ribbing)	[COMP,ASN523LYS]	Ballo et al., 1997	C-to-G transversion	1594	Asn523-to-lys Aa substitution
MED (Fairbanks)	[COMP,ASN453SER]	Briggs et al., 1998	1383A-G transition	Exon 13	Asn453-to-ser (N453S) Aa substitution
PSACH	[COMP,ASP473GLY]	Ikegawa et al., 1998	1418A-G transition		Asp473-to-gly Aa substitution
PSACH	[COMP,(GAC)5,(GAC)7]	Delot et al., 1999	(GAC)5 repeat expansion	1430-1444 cDNA	(GAC)5,(GAC)7
MED (EDM1)	[COMP,(GAC)5,(GAC)6]	Delot et al., 1999	(GAC)5 repeat expansion		(GAC)5,(GAC)6

McKusick (OMIM, http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?132400, 2002) suggested in his discussion of Fairbanks and Ribbing type MED that "... *Fairbanks multiple epiphyseal dysplasia is probably the same as that described as enchondral dysostosis by Odman (1959) and that described as 'microepiphyseal dysplasia' by Elsbach (1959)*...". Clearly, this assumption requires confirmation or

rejection on the basis of further phenotypic and molecular genetic analysis, which is one of the main subjects of this thesis.

EDM2. After the discovery of the COMP gene, a number of other genes involved in the different forms of MED were identified. MED linked to chromosome 1p33-32.2 (EDM2) (Briggs et al., 1994) is also an autosomal dominant form of MED and the gene mutated in a Dutch family with this disorder turned out to be the COL9A2 gene (Muragaki et al., 1996). It encodes the α2 chain of type IX collagen, a structural component of the cartilage extracellular matrix (Warman et al., 1994). Affected individuals typically presented at 2.5 to 6 years of age with pain in the knees. Knee and ankle pain were present throughout childhood.

In some individuals, bilateral osteotomies were required for gross varus deformities of the knees. Hands were mildly short and joints prominent. There were no abnormalities of the spine and chest. Examination of the X-rays showed flattened, irregular epiphyses in most joints, particularly in the knees (Briggs et al., 1994; Barrie et al., 1958). Table II summarizes the currently known allelic variants of EDM2 and associated phenotypes of COL9A2.

Table II. Allelic variants (selected examples) of COL9A2 according to OMIM.

	Allelic variant	Author/year		Site	Result
MED (EDM2)	[COL9A2, IVS3DS, T-C, +2]	Muragaki et al., 1996	Gt-to-Gc transition	Position +2 of intron 3	In-frame loss of 12 amino acids
MED (EDM2)	[COL9A2, G-A, EX3, -1]	Holden et al., 1999	G-to-A transition	Exon 3	CCG(pro) to CCA(pro) in the last codon of exon 3
MED (EDM2)	[COL9A2, IVS3DS, G-C, +5]	Holden et al., 1999	G-to-C transversion	Position +5 of intron 3	
Interverte-bral disc disease	[COL9A2, GLN326TRP]	Annunen et al., 1999	TRP-to-GLN substitution		Tryptophan for glutamine substitution at codon 326

EDM3. A third locus for autosomal dominant MED (EDM3) was found on chromosome 20q13.3 (Paassilta et al., 1999). Subsequent mutation analysis of the positional and functional candidate gene COL9A3 showed an A-to-T conversion at the acceptor splice site of intron 2 of this gene. Bönnemann et al. (2000) described another family with the same form of MED, stressed the importance of myopathy as part of the phenotype, and found – through linkage and mutation analysis and mRNA analysis of a muscle biopsy specimen – also an acceptor splice site mutation of intron 2, leading towards skipping of exon 3. Other variants of this gene seem to be a risk factor in the occurrence of a more common degenerative intervertebral disc disease, as observed by Paassilta et al. (2001) who found an Arg103Trp (R103W) substitution to be associated with disease cases when compared to controls (12.2% versus 4.2%, RR for the allele about three).

EDM4. The fourth type, called EDM4, is responsible for an autosomal recessive form and is caused by a mutation in the DTDST gene on chromosome 5q32-q33.1 (Superti-Furga et al., 1999; Huber et al., 2001; Czarny-Ratajczak et al., 2001). Maroteaux et al. (1975) suggested that the recessive form of MED may differ from the dominant form with respect to the presence of a flat femoral head and lack of metaphyseal irregularities in the metacarpals and phalanges. Chondrocytes contain inclusions with granular or filamentous material, probably of lysosomal origin. One publication stated a homozygous DTDST mutation (R279W) in a male subject, with bilateral clubfoot, surgically corrected in childhood, and a bilateral double-layered patella at 8 years of age (Superti-Furga et al., 1999).

EDM5. The finding of a fifth locus for MED illustrated again the extensive locus heterogeneity of MED. Chapman et al. (2001) described an autosomal

dominant form of MED with knee and hip pain after exercise from early childhood, mild shortness at adult age, multiple epiphyseal dysplasia, early-onset osteoarthritis and a normal spine. Through linkage and mutation analysis they were able to exclude the other loci and to localize the disease on chromosome 2p23-24. Subsequently, the matrilin-3 (MATN-3) gene in this region was identified by Belluoccio et al. (1998) as the one that, if mutated, is causing the disease (two different mutations associated with the disease in two different families).

MATN-3 belongs to a larger group of matrilins that constitute a subfamily of extracellular matrix proteins containing the von Willebrand factor type A (vWFA)-like domain. The vWFA-like domain was first described in von Willebrand factor where it plays a key role in promoting platelet adhesion to the subendothelium. Several vWFA-like domains have been implicated in interactions with collagen. Cartilage matrix protein, also called matrilin-1, and matrilin-2 were the first 2 members of the family to be described.

Chapman et al. (2001) performed a genome wide screen of a 4-generation family with an autosomal dominant form of multiple epiphyseal dysplasia, not linked to COMP, COL9A2 or COL9A3, respectively, and found significant evidence for a MED locus on the short arm of chromosome 2 at 2p23-24. Matrilin-3 was found in the critical region. Chapman et al. (2001) identified two different missense mutations in the exon encoding the von Willebrand factor A domain of MATN-3 in two unrelated families with MED. Table III summarizes the currently known allelic variants of MATN-3 and associated phenotypes, which all show an autosomal dominant pattern of inheritance.

Table III. Allelic variants (selected examples) of multiple epiphyseal dysplasia (MED), MATN-3 related according to OMIM.

	Allelic variant	Author/year		MATN3 gene	Result
MED (EDM5)	[MATN3,VAL194ASP]	Chapman et al., 2001	A-to-T transversion	Position 598	Valine-to-aspartic acid substitution at codon 194
MED (EDM5)	[MATN3,ARG121TRP]	Chapman et al., 2001	C-to-T transversion	Position 378	Arg-to-trp acid substitution at codon 121
MED (EDM5)	[MATN3,ALA128PRO]	Mostert et al., 2003	G-to-C transversion	Position 382 Exon 2	Alanine-to-proline acid substitution at codon 128

3.3. PATHOGENESIS

Many skeletal dysplasias have an unknown aetiology and molecular pathogenesis. In view of the scope of this thesis, special attention will be paid to what is known about the molecular properties of matrilin-3 (MATN-3), a newly discovered member of the novel extracellular matrix protein family.

The matrilin family consists at least of four members (Deák et al., 1999). Whereas MATN-2 and -4 are mainly expressed in non-cartilaginous tissues such as bone and lung, MATN-1 is expressed specifically in the pre-hypertrophic mature zone of a growth plate (Chen et al., 1995). MATN-3 has been found in developing cartilage (Wagener et al., 1997).

All the members of matrilin family contain van Willebrand factor A domains, epidermal growth factor (EGF)-like domains, and a heptad repeat coiled-coil domain at the carboxyl terminus, which is responsible for the oligomerization of the molecule (Beck et al., 1996; Haudenschild et al., 1995).

MATN-1, -2, and -4 contain two A domains (A1 and A2) separated by EGF-like domains, whereas MATN-3 lacks the A2 domain (Wagener et al., 1997). The

A2 domain is near the coiled-coil domain and may modulate oligomerization of matrilins (Zhang and Chen, 2000), whereas the A1 domain is not involved in this oligomer formation process (Chen et al., 1999). MATN-1 contains only one EGF-like domain, whereas MATN-3 contains four such domains. MATN-2 and -4 also contain multiple EGF repeats (Deák et al., 1999).

Among all the matrilins, the position and number of cysteines in every corresponding domain is conserved (Deák et al., 1999). Each A domain has two cysteines, one at the NH_2 terminus and one at the COOH terminus. Each EGF-like domain contains six cysteines. There are two additional cysteines at the NH_2 terminus of the coiled-coil domain. Two such cysteines in MATN-1 (Cys^{455} and Cys^{457}) seem to be responsible for forming intermolecular disulfide bonds linking MATN-1 sub-units together (Haudenschild et al., 1995).

Zhang and Chen (2000) demonstrated that MATN-3 mRNA is mainly expressed in the proliferation zone of a growth plate. It is also expressed in the maturation zone, overlapping with that of the MATN-1 mRNA which is abundant in mature chondrocytes. This suggests that MATN-3 self-assembles in the proliferation zone, as well as co-assembles with MATN-1 during enchondral ossification. Transfection of a MATN-3 cDNA into COS-7 cells shows indeed that MATN-3 predominantly forms a homotetramer but also a trimer and a dimer. Co-transfection of both MATN-3 and MATN-1 cDNAs results in three major matrilins that can be represented in the following formulas: $(MATN\text{-}1)_3$, $(MATN\text{-}3)_4$, and $(MATN\text{-}1)_2(MATN\text{-}3)_2$. Thus, MATN-3 may assemble into both homotypic and heterotypic oligomers. Analysis shows that the assembly of MATN-3 does not depend on the number of epidermal growth factor repeats within the molecule, but the presence of Cys^{412} and Cys^{414} within the coiled-coil domain, which form covalent disulfide linkage responsible for both homo-oligomerization of MATN-3 and hetero-oligomerization of MATN-3 and MATN-1. These data suggest that the

varying synthetic levels of matrilins in different zones of a growth plate may result in a change of matrilin oligomeric forms during enchondral ossification.

Full-length MATN-3 and a fragment lacking the assembly domain were expressed in 293-EBNA cells, purified, and subjected to biochemical characterization. Recombinantly expressed full-length MATN-3 occurs as monomers, dimers, trimers, and tetramers, as detected by electron microscopy and SDS-polyacrylamide gel electrophoresis, whereas MATN-3 purified from fetal calf cartilage, forms homotetramers as well as hetero-oligomers of variable stoichiometry with MATN-1. In the matrix formed by cultured chondrosarcoma cells, MATN-3 is found in a filamentous, collagen-dependent network connecting cells and in a collagen-independent pericellular network.

These findings are in agreement with the observation that fetal growth cartilage contains homotrimeric molecules of MATN-1, homotetrameric molecules of MATN-3, and the assorted chain combinations of MATN-1 and -3 in heterotetramers. In addition, affinity-purified antibodies detect MATN-3 expression in a variety of mouse cartilaginous tissues, such as sternum, articular, and epiphyseal cartilage, and in the cartilage anlage of developing bones. It is found both inside the lacunae and in the interterritorial matrix of the resting, proliferating, hypertrophic, and calcified cartilage zones, whereas the expression is lower in the superficial articular cartilage. In trachea and in costal cartilage of adult mice, an expression was seen in the perichondrium. Furthermore, MATN-3 is found in bone, and its expression is, therefore, not restricted to chondroblasts and chondrocytes.

In a cartilaginous growth plate, extracellular matrix (ECM) molecules mediate cell-matrix and matrix-matrix interactions, thereby providing tissue integrity and a matrix permissible for chondrocyte differentiation and subsequent ossification. Some members of matrilins showed to be expressed specifically in developing

cartilage rudiments but not in adult articular cartilage, suggesting significant differences in temporospatial expression patterns of the genes encoding the various components (Wu and Eyre, 1998; Deák et al., 1999). The prototype of the matrilin family, cartilage matrix protein/MATN-1, has been shown to interact with both collagens (Winterbottom et al., 1992) and aggrecans (Paulsson and Heinegård, 1979). Thus, matrilins may play an important role in the assembly of the ECM networks (Chen et al., 1999).

MATN-2 (Deák et al., 1997; Piecha et al., 1999) and MATN-4 (Wagener et al., 1998 (1); Wagener et al., 1998 (2)) have a much broader tissue distribution than MATN-1 (Paulsson and Heinegård, 1979; Paulsson and Heinegård, 1982; Aszódi et al., 1996) and MATN-3 (Wagener et al., 1997; Belluoccio and Treub, 1997; Belluoccio et al., 1998) which are more restricted to skeletal tissues, but the identification of the co-assembly product, a heterotetramer $(MATN\text{-}1)_2(MATN\text{-}3)_2$, from growth cartilage (Wu and Eyre, 1998) suggested that although the synthesis of the two matrilins are not necessarily spatially identical they do show regional overlap.

The matrilins represent an example of genes identified and functionally analyzed without knowledge of human diseases that may be linked to structural of functional alterations of these genes. The finding of MATN-3 mutations in MED variants including BHMED changed this perspective.

3.4. COURSE AND TREATMENT

There is no specific therapy for MED and MED-related disorders. Problems with MED vary little from problems of normal peers. Early detection is important for purposes of genetic counselling, recreation, and occupation. Affected patients

should be aware that genes causing structurally weak epiphyses may be involved and could be passed on to offspring. Those with weak joints should be warned to avoid recreational or occupational activities particularly traumatic to their joints. Knowledge of the diagnosis and heritable basis of the short-stature conditions might save needless loss of time and expense in hospital visits or admissions, extensive diagnostic work-ups to exclude numerous other causes, and such (Bailey, 1973). The management of MED is also directed towards the prevention of contractures. If they occur in the severe cases, even in the newborn period, they can lead to bony curvatures, angulations, and rotational deformities. As stippling subsides, there is a tendency for contractures to subside, which in turn allows spontaneous correction of secondarily curved or angulated bones. Stretching exercises, sometimes supplemented by plaster casts (usually for foot deformities such as calcaneovalgus feet or clubfoot), are usually adequate. Rotational limb deformities caused by soft tissue also respond to proper positioning (and avoidance of a deforming position), postural and stretching exercises, and casts or braces. Remodelling may be stimulated by continuous passive motion (CPM) of major joints, especially hip and knee (Tachdjian, 1990). The management of epiphyseal dysplasia is also directed towards care of the inevitable osteoarthritis. In severe joint incongruity, axial or rotational deformities of bone with the failure of conservative management, corrective osteotomies, such as pelvic and distal femoral osteotomy, may be indicated and required. Osteotomies performed before closure of the epiphyseal plates gave only palliative correction and the deformities tended to recur (Amir et al., 1985). However, disabling deformities, also in growing children, are an indication for surgery, also when there are loose bodies in the joints. Degenerative osteoarthritis may be treated by total joint arthroplasty.

Patients may also benefit from counselling on body weight and carrier advice. Reduction of late osteoarthritic change may be achieved by restriction of physical

activities in sports and work, and advice on how to take physical exercise (Murphy et al., 1973). Primary prevention is only possible by means of genetic counselling (Maroteaux, 1979).

As in all skeletal dysplasias showing involvement of the spine, the odontoid process should be evaluated as a routine part of the work-up and certainly prior to any surgical procedure. Though only mild to moderate hypoplasia of the odontoid process is the rule, because of the variable nature of the bony disease, an occasional patient shows atlanto-axial instability from severe mal-development. An instability frequency of two in 17 was found in the patients of Afshani and Girdany (1972). Lateral flexion and extension roentgenograms of the upper cervical spine, as well as an open-mouth anteroposterior view, are usually an adequate evaluation. When instability is found, surgical spondylodesis is recommended. If scoliosis develops, treatment of the scoliosis is also necessary.

The course and treatment of MED depends on the severity of the affected and incongruent, arthritic joints. As the patient grows, joint pain and early-onset osteoarthritis develop, sometimes with joint contractures. Early-onset osteoarthritis is more likely when the epiphyseal ossification is fragmented, the femoral head is deformed and poorly covered, and the acetabulum is dysplastic. From the orthopaedic point of view, severe arthritic joints can undergo a joint replacement or in an earlier phase a correction osteotomy, but recurrent deformity is common. Whether osteotomies that are performed to improve biomechanics can alter the natural history of early-onset osteoarthritis is uncertain. A conservative treatment is pursued for as long as possible.

The comparison between the clinical features of the five currently known EDMs is shown in Table IV.

In the autosomal dominant EDM1 type, most complaints are bilateral in the hips and knees.

Shoulder disability is also common, and minor epiphyseal abnormalities can lead to painful osteoarthritis, while severe deformity or 'hatchet head' shoulder result in severe joint limitation at an early age. The hands are clearly involved, with short metacarpals and phalanges and stubby finger ends. Other deformities are scoliosis and valgus ankles, associated with disordered epiphyseal growth. The range of severity of multiple epiphyseal dysplasias is wide, ranging from only local involvement discovered incidentally, to widespread involvement with crippling osteoarthritis and short stature.

In the autosomal dominant EDM2 type, the milder dysplasia, hip and knee complaints are less important although there is a limited joint function and waddling gait. In the study of van Mourik (1998), there were mostly complaints and radiological abnormalities of the knee, elbow, ankle, wrist, hand and foot joints. The shoulders were not involved. Only in two cases the hips showed temporarily slight deformities of the proximal femoral epiphyses. The epiphyses of the major joints were also small and sometimes irregularly shaped. Spine deformities were short stature was explained by these abnormalities, although in the study of van Mourik (1998) the total body height is not significantly different from the control population. The hands and wrists appeared to be unaffected, although brachydactyly was reported.

Table IV. Comparison of the clinical features of the five currently known types of EDM.

	EDM1/COMP/PSACH						EDM2		EDM3		EDM4		EDM5		
	19p13.1						1p33-p32.2		20q13.3		5q32-q33.1		2p23-24		
	Ikegawa et al., 1998	Deere et al., 1999	Deere et al., 1995	Ballo et al., 1997	Weaver et al., 1993	Oehlmann et al., 1994	Briggs et al., 1994	Mourik van, 1998	Paassilta et al., 1999	Bönnemann et al., 2000	Superti-Furga et al., 1999	Gamboa and Lisker, 1974	Chapman et al., 2001	Elsbach, 1959	Mostert et al., 2002
Pedigree generations		3	4	3	5	4		5		4		3	4	4	6
AD/AR					AD		AD	AD			AR	AR	AD	AD	AD
Onset complaints							10					40			4
Bilateral			Y	Y				Y							Y
Early-onset arthritis		Y		Y		Y		Y				Y	Y	Y	Y
Limited joint function	Y			Y				Y		N					Y
Small/irreg. epiphyses	Y		Y		Y										Y
Short stature	Y	Y	Y		Y		Y					Y			Y
TBH			↓				↓						<3%	<3%	<3%
Myopathy								N		Y		N		N	N
Gower sign								N		Y				N	N
Routine lab. abnorm.										Y					N
Short MC IV & V															Y
Short hands			N			Y	Y			N		Y			N
Brachydactyly				Y	Y			N					N	N	N
Ulna plus										N					Y
Complaints elbow										Y					N
X-spine deformities	Y		Y	Y		Y	Y						N	N	N
Limping/waddling gait		Y	Y			Y			Y			Y	Y	Y	Y
Esp. hip complaints			Y			Y		Y	N	N			Y	Y	Y
Valgus femoral neck										N					Y
Short/thick fem. neck			Y							N		Y			Y
Flattened fem. head				Y						N		Y			Y
Acetabulum involved										N					Y
Esp. knee complaints			Y			Y		Y	N	Y			Y	Y	Y
Shallow notch knee			Y			Y				Y			Y		Y
Patella deformity								N			Y			N	N
Sloping distal tibia			Y		Y					Y		Y	Y	Y	Y
Club feet								N			Y				N
Complaints ankle										Y					N

In the autosomal dominant EDM3, mostly complaints of the knees, ankle and elbow are reported. Although there is a waddling gait, no hip abnormalities were reported and the radiological features of the hip were normal. There is a general weakness of the muscles, with for example a positive Gowers' sign and elevated CK levels. No hand deformations are seen. Life expectancy seems to be normal.

The autosomal recessive EDM4 type is a mild dysplasia with a late onset of complaints of the major joints, around 40 years of age. A waddling gait is present and radiologically, the hip shows a flattened head and the collum femoris is shortened and thickened. The bilateral clubfoot, reported by Superti-Furga et al. (1999) is usually corrected in childhood. They also mentioned a bilateral double-layered patella at 8 years of age which can be treated conservatively. Life expectancy seems to be normal.

The autosomal dominant EDM5 is also a clinically variable dysplasia. Hip and knee complaints start in early childhood by frequent falling, difficulty in climbing the stairs and running. When the osteoarthritis of the major joints is severe, joint replacement arthroplasty follows. The shoulder is free of symptoms till the 5th decade. After that, a painful movement develops and radiological symptoms of arthritis can be seen. The other joints, like elbow and ankle show only radiologically small epiphyses, but function and stability are normal. The life expectancy seems to be normal.

In summary, the therapeutic approach is more or less the same for all EDM variants, but systemetic follow-up and comparison is lacking.

Large intra- and interfamilial variation in phenotype, incomplete examination of patients and until recently lack of knowledge about the genes and mutations

involved in MED have hampered the development of a set of reliable features that distinguish the different variants of MED with respect to its diagnosis and prognosis as well its response to different treatments.

It appears that osteoarthritis may complicate any of the currently known EDMs. However, in most reports, the phenotypic description is limited, and follow-up studies providing information about the natural course of disease and effect of treatment are virtually absent.

Social implications

Although there are no specific social implications related to any form of the EDM related families, affected family members show, in case of early-onset osteoarthritis, limitations in their occupation and daily life. These limitations are the same as those in an idiopathic arthritis, without the presence of any kind of dysplasia. It is believed, that heavy weight bearing should be discouraged, based on the joint incongruency or dysplastic development of the different joints. When a joint is poorly covered with articulating surface or when the cartilage is of a lesser quality, it is expected that due to weight bearing the joint is prone to develop an early degeneration, but here the literature is not conclusive.

However, these guidelines are not yet evidence based in view of the absence of adequate follow-up studies that include medical and social endpoints. When the intelligence is normally developed, a regular career can be expected.

The existence of MED in families with an autosomal dominant pattern of inheritance suggests that reproductive fitness is not necessarily reduced in these skeletal dysplasias. Only large population-based studies will enable us to make more precise conclusions about marriage rates and fertility in male and female patients. Even if reproductive fitness werebe undisturbed, one may expect that MED variants characterized by shortened stature may lead towards height-related

partner choice and thus continued gene influx from a polygenetic background for short stature in subsequent generations of familial autosomal dominant MED. This issue will also be addressed in this thesis.

REFERENCES

Afshani E, Girdany BR. Atlanto-axial dislocation in chondrodysplasia punctata. Radiology 1972;102:399–401.

Ahmad NN, Ala-Kokko L, Knowlton RG, Weaver EJ, Jimenez SA, Maguire JI, Tasman W, Prockop DJ. Stop codon in the gene for type II procollagen (COL2A1) in a family with the Stickler syndrome (arthro-ophthalmopathy). Proc Natl Acad Sci USA 1991;88: 6624–6627.

Ahmad NN, McDonald-McGinn DM, Zackai EH, Knowlton RG, LaRossa D, Dimascio J, Prockop DJ. A second mutation in the type II procollagen gene (COL2A1) causing the Stickler syndrome (arthro-ophthalmopathy) is also a premature termination codon. Am J Hum Genet 1993;53:39–45.

Ala-Kokko L, Baldwin CT, Moskowitz RW, Prockop DJ. Single base mutation in the type II procollagen gene (COL2A1) as a cause of osteoarthritis associated with mild chondrodysplasia. Proc Natl Acad Sci USA 1990;87:6565–6568.

Amir D, Mogle P, Weinberg H. Multiple epiphyseal dysplasia in one family. A further review of seven generations. J Bone Joint Surg 1985;67B:809–813.

Annunen S, Paassilta P, Lohiniva J, Perala M, Pihlajamaa T, Karppinen J, Tervonen O, Kroger H, Lahde S, Vanharanta H, Ryhanen L, Goring HHH, Ott J, Prockop DJ, Ala Kokko L. An allele of COL9A2 associated with Intervertebral disk disease. Science 1999;285:409–412.

Apley AG, Solomon L. Apley's System of Orthopadcs and Fractures, 7th edition. Oxford: Butterworth-Heinemann Ltd., 1993.

Aszódi A, Hauser N, Studer D, Paulsson M, Hiripi L, Bösze Z. Cloning, sequencing and expression analysis of mouse cartilage matrix protein cDNA. Eur J Biochem 1996;236(3):970–977.

Bachman K, Norman AP. Hereditary peripheral dysostosis (3 cases). Proc Roy Soc Med 1967;60:21–22.

Bailey JA II. Disproportionate Short Stature: Diagnosis and Management. Philadelphia, PA, WB Saunders, 1973.

Ballo R, Briggs MD, Cohn DH, Knowlton RG, Beighton PH, Ramesa RS. Multiple epiphyseal dysplasia, ribbing type: a novel point mutation in the COMP gene in a South African family. Am J Med Genet 1997;68(4):396–400.

Barrie H, Carter C, Sutcliffe J. Multiple epiphyseal dysplasia. Br Med J 1958;2:133-137.

Barrington-Ward LE: Double coxa vara with other deformities occurrring in brother and sister. Lancet 1912;i:157.

Beals R, Horton W. Skeletal dysplasias: An approach to diagnosis. J Am Acad Orthop Surg 1995;3:174–181.

Beck K, Gambee JE, Bohan CA, Bachinger HP. The C-terminal domain of cartilage matrix protein assembles into a triple stranded alpha-helical coiled-coil structure. J Mol Biol 1996;256(5):909–923.

Belluoccio D, Schenker T, Baici A, Trueb B. Characterization of human matrilin-3 (MATN3). Genomics 1998;53(3):391–394.

Belluoccio D, Trueb B. Matrilin-3 from chicken cartilage. FEBS Lett 1997;415(2):212–216.

Bogaert R, Tiller GE, Weis MA, Gruber HE, Rimoin DL, Cohn DH, Eyre DR. An amino acid substitution (gly853→glu) in the collagen alpha 1(II) chain produces hypochondrogenesis. J Biol Chem 1992;267:22522–22526.

Bönnemann CG, Cox GF, Shapiro F, Wu JJ, Feener CA, Thompson TG, Anthony DC, Eyre DR, Darras BT, Kunkel LM. A mutation in the alpha-3 chain of type IX collagen causes autosomal dominant multiple epiphyseal dysplasia with mild myopathy. Proc Natl Acad Sci USA 2000;97(3):1212–1217.

Breck LW. An Atlas of the Osteochondroses. Springfield, Charles C. Thomas, 1971.

Briggs MD, Choi H, Warman ML, Loughlin JA, Wordsworth P, Sykes BC, Irven CMM, Smith M, Wynne-Davies R, Lipson MH, Biesecker LG, Garber AP, Lachman R, Olsen BR, Rimoin DL, Cohn DH. Genetic mapping of a locus for multiple epiphyseal dysplasia (EDM2) to a region of chromosome 1 containing a type IX collagen gene. Am J Hum Genet 1994;55:678–684.

Briggs MD, Hoffman SMG, King LM, Olsen AS, Mohrenweiser H, Leroy JG, Mortier GR, Rimoin DL, Lachman RS, Gaines ES, Cekleniak JA, Knowlton RG, Cohn DH. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nature Genet 1995;10:330–336.

Briggs MD, Mortier GR, Cole WG, King LM, Golik SS, Bonaventure J, Nuytinck L, De Paepe A, Leroy JG, Biesecker L, Lipson M, Wilcox WR, Lachman RS, Remoin DL, Knowlton RG, Cohn DH. Diverse mutations in the gene for cartilage oligomeric matrix protein in the pseudoachondroplasia-multople epiphyseal dysplasia disease spectrum. Am J Hum Genet 1998;62(2):311–319.

Brown DM, Nichols BE, Weingeist TA, Sheffield VC, Kimura AE, Stone EM. Procollagen II gene mutation in Stickler syndrome. Arch Ophthalmol 1992;110:1589–1593.

Byers PH. Brittle bones-fragile molecules: disorders of collagen gene structure and expression. Trends Genet 1990;6:293–300.

Cameron JM, Gardiner TB. Atypical familial osteochondrodystrophy. Br J Radiol 1963;36:135–139.

Chan D, Cole WG. Low basal transcription of genes for tissue-specific collagens by fibroblasts and lymphoblastoid cells. J Biol Chem 1991;266:12487-12494.

Chapman KL, Mortier GR, Chapman K, Loughlin J, Grant ME, Briggs MD. Mutations in the region encoding the von Willebrand factor A domain of matrilin-3 are associated with multiple epiphyseal dysplasia. Nature Genet 2001;28(4):393–396.

Chen Q, Johnson DM, Haudenschild DR, Goetinck PF. Progression and recapitulation of the chondrocyte differentiation program: cartilage matrix protein is a marker for cartilage maturation. Dev Biol 1995;172(1):293–306.

Chen Q, Zhang Y, Johnson DM, Goetinck PF. Assembly of a novel cartilage matrix protein filamentous network: molecular basis of differential requirement of von Willebrand factor A domains. Mol Biol Cell 1999;10(7):2149–2162.

Crossan JF, Wynne-Davies R, Fulford GE. Bilateral failure of the capital femoral epiphysis: bilateral Perthes' disease, multiple epiphyseal dysplasia, pseudoachondroplasia, and spondyloepiphyseal dysplasia congenita and tarda. J Pediatr Orthop 1983;3:297–301.

Czarny-Ratajczak M, Lohiniva J, Rogala P, Kozlowski K, Perala M, Carter L, Spector TD, Kolodziej L, Seppanen U, Glazar R, Krolewski J, Latos-Bielenska A, Ala-Kokko L. A Mutation in COL9A1 causes multiple epiphyseal dysplasia: further evidence for locus heterogeneity. Am J Hum Genet 2001;69(5): 969–980.

Deák F, Piecha D, Bachrati C, Paulsson M, Kiss I. Primary structure and expression of matrilin-2, the closest relative of cartilage matrix protein within the von Willebrand factor type A-like module superfamily. J Biol Chem 1997;272(14):9268–9274.

Deák F, Wagener R, Kiss I, Paulsson M. The matrilins: a novel family of oligomeric extracellular matrix proteins. Matrix Biol 1999;18(1):55–64.

Dee R. Principles of orthopaedic practice. 2nd Edition. The McGraw-Hill Companies Inc. New York, 1997.

Deere M, Blanton SH, Scott CI, Langer LO, Pauli, RM, Hecht, JT. Genetic heterogeneity in multiple epiphyseal dysplasia. Am J Hum Genet 1995;56:698-704.

Deere M, Sanford T, Francomano CA, Daniels K, Hecht JT. Identification of nine novel mutations in cartilage oligomeric matrix protein in patients with pseudoachondroplasia and multiple epiphyseal dysplasia. Am J Med Genet 1999;85(5):486–490.

Delot E, King LM, Briggs MD, Wilcox WR, Cohn DH. Trinucleotide expansion mutations in the cartilage oligomeric matrix protein (COMP) gene. Hum Mol Genet 1999; 8(1):123–128.

Diamond LS. A family study of spondyloepiphyseal dysplasia. J Bone Joint Surg 1970;52B:1587–1594.

Elsbach L. Bilateral hereditary miro-epiphyseal dysplasisa of the hips. J Bone Joint Surg 1959;41B:514–523.

Emr J. Dysplasia epiphysialis multiplex occurring in two brothers. Acta Chir Orthop Traumatol Cech 1952;19:310.

Fairbank HAT. Dysplasia epiphysialis multiplex. Br J Surg 1947;34:225–232.

Fairbank HAT. Generalized Diseases of the Skeleton. Procedings of the Royal Society of Medicine (Clinical Section) 1935;28:6111–1619.

Fryns JP, van den Berghe H. An asymmetric type of chondrodysplasia in an adult male: another example of postzygotic mutation for an autosomal dominant gene? Clin Genet 1986;30:324–327.

Gamboa I, Lisker R. Multiple epiphyseal dysplasia tarda. A family with autosomal recessive inheritance. Clin Genet 1974;6(1):15–19.

Hall JG, Dorst JP, Rotta J, Mc Kusick VA. Gonadal mosaicism in pseudoachondroplasia. Am J Med Genet 1987;28:143–151.

Hall CM. International Nosology and classification of constitutional disorders of bone (2001). Am J Med Genet 2002;113(1):65–77.

Haudenschild DR, Tondravi MM, Hofer U, Chen Q, Goetinck PF. The role of coiled-coil alpha-helices and disulfide bonds in the assembly and stabilization of cartilage matrix protein subunits. J Biol Chem 1995;270(39):23150–23154.

Hecht JT, Nelson LD, Crowder E, Wang Y, Elder FFB, Harrison WR, Francomano CA, Prange CK, Lennon GG, Deere M, Lawler J. Mutations in exon 17B of cartilage oligomeric matrix protein (COMP) cause pseudoachondroplasia. Nature Genet 1995;10:325–329.

Hoback A. Problems of Hereditary Chondrodysplasias. Oslo, Oslo University Press, 1961.

Hoefnagel D, Sycamore LK, Russell SW, Bucknall WE. Hereditary multiple epiphyseal dysplasia. Ann Hum Genet 1967;30:201–210.

Holden P, Canty EG, Mortier GR, Zabel B, Spranger J, Carr A, Grant ME, Loughlin JA, Briggs MD. Identification of a novel pro-alpha-2(IX) collagen gene mutations in two families with distinctive oligo-epiphyseal forms of multiple epiphyseal dysplasia. Am J Hum Genet 1999;65:31–38.

Horton WA, Machado MA, Ellard J, Campbell D, Bartley J, Ramirez F, Vitale E, Lee B. Characterization of a type II collagen gene (COL2A1) mutation identified in cultured chondrocytes from human hypochondrogenesis. Proc Natl Acad Sci USA 1992;89: 4583–4587.

Huber C, Odent S, Rumeur S, Padovani P, Penet C, Cormier-Daire V, Munnich A, Le Merrer M. Sulphate transporter gene mutations in apparently isolated club foot. J Med Genet 2001;38(3):191–193.

Hulvey JT, Keats TE. Multiple epiphyseal dysplasia: a contribution to the problem of spinal involvement. Am J Roentgen 1969;106:170–177.

Ikegawa S, Iwaya T, Taniguchi K, et al: Retinal detachment in spondyloepiphyseal dysplasia congenita. J Pediatr Orthop 1993;13:791–792.

Ikegawa S, Ohashi H, Nishimura G, Kim KC, Sannohe A, Kimizuka M, Fukushima Y, Nagai T, Nakamura Y. Novel and recurrent COMP (cartilage oligomeric matrix protein) mutations in pseudoachondroplasia and multiple epiphyseal dysplasia. Hum Genet 1998;103(6):633–638.

Ikegawa S. Spondyloepiphyseal dysplasia tarda: The autosomal recessive form in two sisters. Arch Orthop Trauma Surg 1993;113:49–52.

Ingram RR. Early diagnosis of multiple epiphyseal dysplasia. J Pediatr Orthop 1992;12(2):241–244.

Ingram RR. The shoulder in multiple epiphyseal dysplasia. J Bone Joint Surg 1991;73B:277–279.

Juberg RC, Holt JF. Inheritance of multiple epiphyseal dysplasia tarda. Am J Hum Genet 1968;20:549–563.

Knowlton RG, Cekleniak JA, Cohn DH, Briggs MD, Hoffman, SMG, Brandriff BF, Olsen AS. High-resolution genetic and physical mapping of multiple epiphyseal dysplasia and pseudoachondroplasia mutations at chromosome 19p13.1-p12. Genomics 1995;28:513–519.

Kopits SE, Lindstrom JA, Mc Kusick VA. Pseudoachondroplastic dysplasia: patho-dynamics and management. In Bergsma D: Skeletal dysplasias. Amsterdam: Excerpta Medica (publ), 1974;341–352.

Langer LO Jr, Schaefer GB, Wadsworth DT. Patient with double heterozygosity for achondroplasia and pseudoachondroplasia, with comments on these conditions and the relationship between pseudoachondroplasia and multiple epiphyseal dysplasia, Fairbank type. Am J Med Genet 1993;47:772–781.

Lee B, Vissing H, Ramirez F, Rogers D, Rimoin D. Identification of the molecular defect in a family with spondyloepiphyseal dysplasia. Science 1989;244:978–980.

Leeds NE. Epiphyseal dysplasia multiplex. Am J Roentgenol 1960;84:506–510.

Litchman HM, Chirls M. Dysplasia epiphysealis multiplex. Bull Hosp Joint Dis 1958;19:88–99.

Lodes R. Familiäres Vorkommen habitueller bilateraler luxation zweigeteiler Kniescheiben bei Chondrodystrophy. Z Orthop 1949;78:506–524.

Maroteaux P, Stanesccu R, Cohen-Solal D. Poly-epiphyseal dysplasia, probably autosomal recessive. Contribution of the ultrastructural study to the discovery of this autonomous form. Nouv Presse Med 1975;4(30):2169–2172.

Maroteaux P. Epiphyseal dysplasia, multiple. In: Bergsma DR. Birth Defects Compendium. New York, National Foundation, March of Dimes: Alan R Liss, New York, 1979;409.

Maroteaux P. Spondyloepiphyseal dysplasias and metatropic dwarfism. In: Birth Defects: Original Article Series 1969;5:35–41.

Mc Keand J, Rotta J, Hecht JT. Natural history study of pseudoachondroplasia. Am J Med Genet 1996;63:406–410.

Meyer J. Dysplasia epiphysealis capitis femoris. A clinical-radiological syndrome and its relationship to Legg-Calvé-Perthes' disease. Acta Orthop Scand 1964;34:183–197.

Mostert AK, Dijkstra PF, Jansen BRH, van HornJR, de Graaf B, Heutink P, Lindhout D. Familial multiple epiphyseal dysplasia due to a matrilin-3 mutation. Further delineation of the phenotype including 40 years follow-up. Am J Med Genet 2003;in press.

Mostert AK, Jansen BR, Dijkstra PF, Wesby-Van Swaay B, Van Horn JR, Heutink P, Lindhout D. Bilateral hereditary micro-epiphyseal dysplasia: Further delineation of the phenotype with 40 years follow-up. Int Orthop 2002;26:188–193.

Mourik van JBA, Schaap C, Nollen AJG. Een Nederlandse familie met erfelijke gewrichtsklachten; multipele epifysaire dysplasie. Ned Tijdschr Geneeskd 1993;137:32–36.

Mourik van JBA. Multiple Epiphyseal Dysplasia. A clinical and molecular genetic study. Thesis 1998, Rotterdam.

Muragaki Y, Mariman ECM, van Beersum SEC, Perala M, van Mourik JBA, Warman ML, Olsen BR, Hamel BCJ. A mutation in the gene encoding the alpha-2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2). Nature Genet 1996;12:103–105.

Murphy MC, Shine I, Stevens DB. Multiple epiphyseal dysplasia: report of a pedigree. J Bone Joint Surg 1973;55A:814–820.

Odman P. Hereditary enchondral dysostosis: twelve cases in three generations mainly with peripheral location. Acta Radiol 1959;52:97–113.

Oehlmann R, Summerville GP, Yeh G, Weaver EJ, Jimenez SA, Knowlton RG. Genetic linkage mapping of multiple epiphyseal dysplasia to the pericentromeric region of chromosome 19. Am J Hum Genet 1994;54:3–10.

Oehlmann R, Yeh G, Summerville GP, Weaver EJ, Jimenez SA, Knowlton RG. Multiple epiphyseal dysplasia maps to chromosome 19. (Abstract) Am J Hum Genet 1993;53 (suppl): A1054 only.

Paassilta P, Lohiniva J, Annunen S, Bonaventura J, Le Merrer M, Pai L, Ala-Kokko L. COL9A3: A third locus for multiple epiphyseal dysplasia. Am J Hum Genet 1999;64(4):1036–1044.

Paassilta P, Lohiniva J, Goring HH, Perala M, Maina SS, Karppinen J, Hakala M, Palm T, Kroger H, Kaitila I, Vanharanta H, Ott J, Ala-Kokko L. Identification of a novel common genetic risk factor for lumbar disk disease. JAMA 2001;285(14):1843–1849.

Paulsson M, Heinegård D. Matrix proteins bound to associatively prepared proteoglycans from bovine cartilage. Biochem J 1979;183(1):539–545.

Paulsson M, Heinegård D. Radioimmunoassay of the 148-kilodalton cartilage protein. Distribution of the protein among bovine tissues. Biochem J 1982;207(2):207–213.

Piecha D, Muratoglu S, Mörgelin M, Hauser N, Studer D, Kiss I, Paulsson M, Deák F. Matrilin-2, a large, oligomeric matrix protein, is expressed by a great variety of cells and forms fibrillar networks. J Biol Chem 1999;274(19):13353–13361.

Poznanski AK, Garn SM, Nagy JM, Gall JC Jr. Metacarpophalangeal pattern profiles in the evaluation of skeletal malformations. Radiology 1972:104(1);1–11.

Ribbing S. Studien über Hereditäre, Multiple Epiphysen-Störungen. Acta Radiol (Suppl) 1937;34.

Rimoin DL, Lachman RS. Genetic disorders of the osseous skeleton. In: Beighton P (ed): McKusick's Heritable Disorders of Connective Tissue, 5th Edition. St. Louis, Mosby, 1993;622–625.

Rimoin DL, Rasmussen IM, Briggs MD, Roughley PJ, Gruber HE, Warman ML, Olsen BR, Hsai YE, Yuen J, Reinker K, Garber AP, Grover J, Lachman RS, Cohn DH. A large family with features of pseudoachondroplasia and multiple epiphyseal dysplasia: exclusion of seven candidate gene loci that encode proteins of the cartilage extracellular matrix. Hum Genet 1994;93:236–242.

Rubin P. Dynamic Classification of Bone Dysplasias. Chicago, IL, Year Book Medical Publishers, 1964.

Silverman FN. A differential diagnosis of achondroplasia. Radiol Clin North Am 1968;6(2):223–237.

Spranger J. Pattern recognition in bone dysplasias. In: Papadotos C, Bartsocas C (eds) Endocrine genetics and genetics of growth. Alan R Liss, New York, 1985;315.

Spranger J. The epiphyseal dysplasias. Clin Orthop 1976;114:46–59.

Stanescu R, Stanescu V, Muriel M-P, Maroteaux P. Multiple epiphyseal dysplasia, Fairbanks type: morphologic and biochemical study of cartilage. Am J Med Genet 1993;45:501–507.

Stanescu V, Maroteaux P, Stanescu R. The biochemical defect of pseudoachondroplasia. Eur J Pediat 1982;138:221–225.

Superti-Furga A, Neumann L, Riebel T, Eich G, Steinmann B, Spranger J, Kunze J. Recessively inherited multiple epiphyseal dysplasia with normal stature, club foot, and double layered patella caused by a DTDST mutation. J Med Gen 1999;36:621–624.

Tachdjian MO. Pediatric orthopaedics, 2nd edition. Philadelphia: WB Saunders Company, 1990.

Tiller GE, Rimoin DL, Murray LW, Cohn DH. Tandem duplication within a type II collagen gene (COL2A1) exon in an individual with spondyloepiphyseal dysplasia. Proc Natl Acad Sci USA 1990;87:3889–3893.

Treble NJ, Jensen FO, Bankier A, Gogers JG, Cole WG. Development of the hip in multiple epiphyseal dysplasia. Natural history and susceptibility to premature osteoarthritis. J Bone Joint Surg 1990;72B:1061–1064.

Vikkula M, Ritvaniemi P, Vuorio AP, Kaitila I, Ala-Kokko L, Peltonen L. A mutation in the amino-terminal and of the triple helix of type II collagen causing severe osteochondrodysplasia. Genomics 1993;16:282–285.

Villareal T, Carnevale A, Mayen DG, Takenaga R, del Castillo, V. Anthropometric studies in five children and their mother with a severe form multiple epiphyseal dysplasia. Am J Med Genet 1992;42:415–419.

Vissing H, d'Alessio M, Lee B, Ramirez F, Godfrey M, Hollister DW. Glycine to serine substitution in the triple helical domain of pro-alpha 1(II) collagen results in a lethal perinatal form of short-limbed dwarfism. J Biol Chem 1989;264:18265–18267.

Wagener R, Kobbe B, Paulsson M. Genomic organization, alternative splicing and primary structure of human matrilin-4. (2) FEBS Lett 1998;438(3):165–170.

Wagener R, Kobbe B, Paulsson M. Matrilin-4, a new member of the matrilin family of extracellular matrix proteins. (1) FEBS Lett 1998;486(3):123–127.

Wagener R, Kobbe B, Paulsson M. Primary structure of matrilin-3, a new member of a family of extracellular matrix proteins related to cartilage matrix protein (matrilin-3) and von Willebrand factor. FEBS Lett 1997;413(1):129–134.

Warman ML, McCarthy MT, Perala M, Vuorio E, Knoll JHM, McDaniels CN, Mayne R, Beier DR, Olsen BR. The genes encoding alpha-2(IX) collagen (COL9A2) map to human chromosome 1p32.3-p33 and mouse chromosome 4. Genomics 1994;23:158–162.

Watt JK. Multiple epiphyseal dysplasia: report of four cases. Br J Surg 1952;39:533–535.

Waugh W. Displasia epiphysialis multiplex in 3 sisters. J Bone Joint Surg 1952;34B:82.

Weaver EJ, Summerville GP, Yeh G, Hervada-Page M, Oehlmann R, Rothman R, Jimenez SA, Knowlton RG. Exclusion of type II and type IV procollagen gene mutations in a five-generation family with multiple epiphyseal dysplasia. Am J Med Genet 1993;45:345–352.

Winterbottom N, Tondravi MM, Harrington TL, Klier FG, Vertel BM, Goetinck PF. Cartilage matrix protein is a component of the collagen fibril of cartilage. Dev Dyn 1992;193(3):266–276.

Winterpacht A, Hilbert M, Schwarze U, Mundlos S, Spranger J, Zabel BU. Kniest and Stickler dysplasia phenotypes caused by collagen type II gene (COL2A1) defect. Nature Genet 1993;3:323–326.

Wu JJ, Eyre DR. Matrilin-3 forms disulfide-linked oligomers with matrilin-1 in bovine epiphyseal cartilage. J Biol Chem 1998;273(28):17433-17438.

Wynne-Davies R, Gormley J. The prevalence of skeletal dysplasias. An estimate of their minimum frequency and the number of patients requiring orthopaedic care. J Bone Joint Surg 1985;67B:133–137.

Wynne-Davies R, Hall C, Ansell B. Spondyloepiphyseal dysplasia tarda with progressive arthropathy. A 'new' disorder of autosomal recessive inheritance. J Bone Joint Surg 1982;64B:442–445.

Wynne-Davies R, Hall C. Two clinical variants of spondyloepiphyseal dysplasia congenita. J Bone Joint Surg 1982;64B:435–441.

Zhang Y, Chen Q. Changes of matrilin forms during endochondral ossification. Molecular basis of oligomeric assembly. J Biol Chem 2000;275(42):32628–32634.

CHAPTER 4

THE STUDY

4.1. BILATERAL HEREDITARY MICRO-EPIPHYSEAL DYSPLASIA

Further delineation of the phenotype with 40 years follow-up

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ABSTRACT

Bilateral Hereditary Micro-Epiphyseal Dysplasia (BHMED) is a distinct entity in the group of skeletal dysplasias on the basis of specific clinical and radiological findings. The family originally published by Elsbach [5] in 1959 was re-examined for further delineation of the phenotype. Especially the hip and knee were affected bilaterally and simultaneously with small epiphyses and osteoarthritis at an early age. In addition, some subjects showed shortening of metacarpals IV and V. A small adult height, associated with BHMED, proved to be comfounded in this family by height related partner choice. Future linkage analysis and characterisation of the molecular defect causing BHMED will clarify whether this disorder is allelic to other known skeletal dysplasias like multiple epiphyseal dysplasia, or is due to a mutation of a novel gene.

INTRODUCTION

Bilateral hereditary micro-epiphyseal dysplasia (BHMED) is a rare, usually symmetrical skeletal dysplasia first reported by Elsbach [5] in 1959. In the family he described, several members complained mainly of pain in hips and knees from early childhood.

Because of the radiological finding of small epiphyses in the hip and knee joint, Elsbach designated the disorder bilateral hereditary micro-epiphyseal dysplasia. BHMED should be differentiated from multiple epiphyseal dysplasia (MED), in which the epiphyses have a (relatively) normal size.

Recently, several members of the original family were referred to the department of orthopaedic surgery in Delft with questions about the genetic

background, and the recurrence risk. We re-examined the extended family, in order to evaluate diagnostic criteria and the clinical course, to determine the clinical spectrum, and to provide tools for the diagnosis of possibly affected persons with only partial expression of the disease.

PATIENTS AND METHODS

Clinical examination. The pedigree was updated and contained 54 subjects in six generations (Fig. 1). Thirty-two members of the family and 12 spouses were examined by the first author (A.K.M.). A full medical history was taken according to a standardised questionnaire. Physical examination was performed according to a standard protocol. The range of motion of hip, knee, and shoulder joints was measured according to guidelines (4th edition) of the American Medical Association [4]. Total body height, sitting height, leg length, and span width were measured and compared with reference data from the Dutch population [2].

Prior and additional radiographs of first- and second-degree family members complaints were evaluated by one of the authors (P.F.D.). In some of the cases, results of laboratory tests of serum calcium, phosphate and alkaline phosphatase levels were available and collected.

Diagnostic criteria. All subjects over 4 years old were classified clinically and radiologically as affected, unaffected, or inconclusive (included not known).

Clinical classification. The original clinical description by Elsbach [5] was used as the basis for the clinical diagnosis of BHMED. Diagnostic criteria for clinical affection status were bilateral simultaneous onset of pain in hip and knee joints, short stature, waddling gait and restricted range of motion of the hip. Patients and

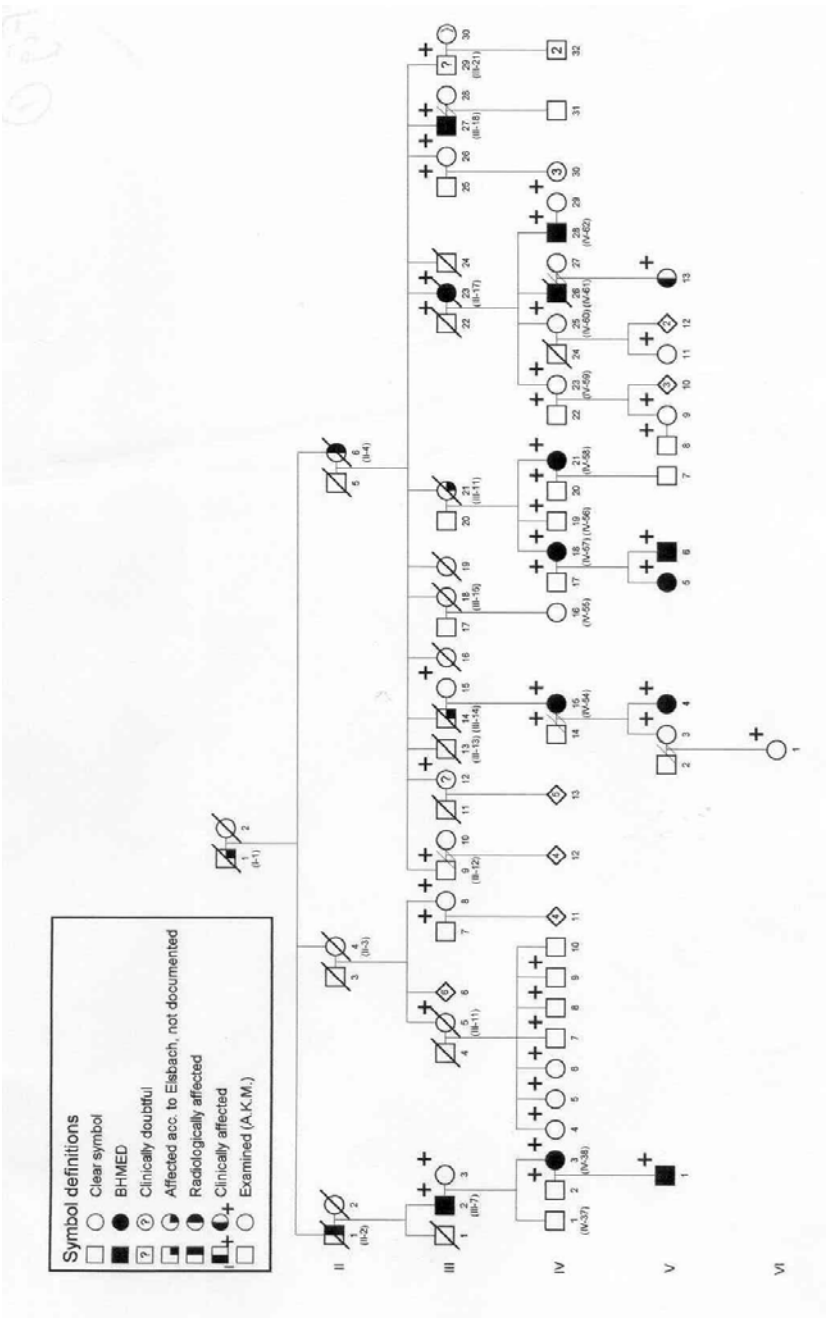

Figure 1. Revised pedigree of the BHMED family. Identification numbers between brackets represent dentification numbers in the pedigree originally published by Elsbach (1959) [5].

relatives who fulfilled all clinical diagnostic criteria were categorised as affected (group I). Subjects who did not fulfil all diagnostic criteria, or who were not examined or only incompletely examined were categorised as inconclusive with respect to clinical affection status (group II). Patients who showed no clinical signs of BHMED were categorised as unaffected (group III).

Radiological classification. All radiographs were reviewed by a radiologist specialised in skeletal dysplasias (P.F.D.), without knowledge of the clinical evaluation or the a priori risk for the condition. The diagnostic criteria for radiological affection status were (a) a hip characterised by valgus angle of the femoral neck [8] which is short and thickened [17]; (b) flattening of the head of the femur especially at the medial part, dysplasia of the acetabulum by an apparently abnormal teardrop configuration of the medial acetabular wall (Fig. 2); (c) small epiphyses of proximal and distal femur and proximal tibia with or without flattened condyles of the knees and a shallow intercondylar notch; and (d) a symmetrical distribution of abnormalities. Subjects fulfilling the first, second, and fourth criterion (minimal diagnostic criteria) were regarded as radiologically affected. Patients not fulfilling these criteria were classified as radiologically unaffected.

One of the two index patients of the publication by Elsbach had died before the re-examination (Fig. 1, IV-26). This was the only patient we regarded as affected on the basis of the clinical and radiological description by Elsbach only.

Genetic examination. We used all members of the family for whom DNA was available.

RESULTS

Pedigree. The updated pedigree (Fig. 1) shows only those subjects of whom information about the phenotype was available.

In 6 successive generations, 15 subjects were clinically and/or radiologically affected. The male/female ratio among patients was close to 1. This pattern of familial occurrence and possibly one male to male transmission (II-1→III-2) suggest an autosomal dominant mode of inheritance. All subjects with an affected parent and at least one affected child, and who had been examined clinically and radiologically, showed clinical expression, suggesting that at adult age the penetrance of the gene defect is high.

Clinical findings. Six men – five according to the clinical criteria, and one on the basis of Elsbach's description [5] – and eight women were classified as clinically affected (group I); for one male and one female at 50% prior risk, the clinical affection status was inconclusive (group II), whereas in two men and eight women at 50% prior risk, the diagnosis of BHMED was clinically excluded (group III) (Table I).

Table I. Summary of clinical affection status.

Clinical category	Male	Female	Total
Group I (Affected)	6	8	14
Group II (Inconclusive)	1	1	2
Group III (Unaffected)	2	8	10
Total	9	17	26

Table II. Summary of radiological versus clinical affection status.

Radiological affection	Clinical affection status				
	Affected	Unaffected	Doubtful	Unknown	Total
Affected	13	-	-	2*	15
Unaffected	1	9	-	-	10
Doubtful**	-	-	1	-	1
Unknown**	-	1	1	-	2
Total	14	10	2	2	28

* This table includes two patients with radiographs previously published by Elsbach (1959) [5] but without clinical re-examination in the current study.

** Inconclusive is split up into doubtful and not known (see section methods/diagnostic criteria).

The clinically affected subjects (group I) clearly showed reduced femur abduction (limited with an average of 15°) and internal rotation (limited with an average of 25°) as compared to group III (unaffected), whereas group II (inconclusive) is too small and heterogeneous for meaningful comparison. There was no loss of knee mobility among patients of group I, although all persons classified clinically as affected suffered from knee complaints. The mobility of the shoulders was unlimited, even at older age, although they were painful above the age of 50 in group I. The hands showed no malformations. However, a shortening of the metacarpals (MC), especially of MC IV and V, became more apparent upon clenching of the fists.

Radiological findings. The six men and eight women who were classified as affected according to clinical criteria (group I) were also classified as affected according to radiological criteria, except one. This woman fulfilled the clinical diagnostic criteria, but the radiographs were considered normal (Table II). The remaining three men and nine women were radiologically classified as unaffected or inconclusive.

Figure 2. AP-radiograph of the pelvis and hips of a BHMED patient (IV-15).

The hip joint showed a valgus angle of the femoral neck which is short and thickened, flattening of the head of the femur especially at the medial part, and a dysplasia of the acetabulum by an apparently abnormal teardrop configuration of the medial acetabular wall (Fig. 2).

Additional radiographic features observed more than once among persons classified as radiologically affected were a shallow intercondylar notch on the AP view (Fig. 3A) and small epiphyses and flattened condyles on the lateral view of the knee (Fig. 3B). In a few cases the lateral femoral condyle showed a focus of osteochondritis dissecans or an ulcus. Radiographs of the wrist from affected relatives showed an 'ulna plus' with slight radial drift of the carpus and flattening

of the ulnar part of the radial epiphysis (Fig. 4). The spine showed signs of lumbar spondylosis, whereas the cervical and thoracic part of the spine was normal. Flattened vertebrae, such as occur in e.g. spondylo-epiphyseal dysplasia [6], were not observed.

Body measurements. Patients who were classified as clinically and radiologically affected had a lower mean total body height (TBH) in comparison with unaffected first-degree relatives (Table III). However, the difference was larger when the affected persons were compared with controls from the out-patients clinic or with the reference population. Compared to controls, also the unaffected first-degree relatives of BHMED patients showed a lower total body height. Among men, a similar trend is observed for sitting height and span width.

Figure 3. Radiographs of the right knee of a BHMED patient (III-2) (Fig. 3A, lateral view; Fig. 3B, AP view).

Figure 4. PA-radiograph of the wrist (IV-3) with an 'ulna plus' with slight radial drift of the carpus and flattening of the ulnar part of the radial epiphysis.

In turn, also unaffected first-degree relatives of patients showed a reduced total body height as compared to the general population, matched for age and gender.

Laboratory data. Available results from additional laboratory measurements including calcium, phosphate and alkaline phosphatase levels in serum were within the normal ranges.

Table III. Body measurements in BHMED patients compared with controls and reference population (values in cm).

		Total body height				Sitting height				Leg length				Span width			
				Range				Range				Range				Range	
Group	N	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Men																	
Affected men	5	164	7	154	171	87	4	81	91	76	3	73	80	166	7	157	174
Unaffected 1st degree male relatives	2	165	3	163	167	86	2	84	87	80	1	79	80	160	3	158	162
Male spouses of affected women	5	168	5	160	173	90	4	84	93	78	6	72	89	170	6	163	179
Male spouses of unaffected women	4	178	3	175	182	95	3	91	98	83	3	79	87	179	5	172	182
Control men (outpatient clinic)	21	178	10	160	195	92	11	58	106	86	10	72	120	179	10	160	196
Control men (Dutch population)	10843	179.7	10.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Women																	
Affected women	8	154	4	148	159	81	2	78	84	83	3	79	87	155	5	148	161
Unaffected 1st degree female relatives	7	165	5	155	172	85	3	80	90	80	4	75	85	167	5	160	173
Female spouses of affected men	2	162	0	162	162	89	1	88	89	74	1	73	74	162	1	161	163
Female spouses of unaffected men	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control women (outpatient clinic)	31	166	7	155	188	90	5	82	101	75	5	64	87	167	8	150	183
Control women (Dutch population)	11178	167.2	10.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

In this study we analysed the clinical and radiological features of a skeletal dysplasia previously described by Elsbach in 1959. Elsbach allocated the name bilateral hereditary micro-epiphyseal dysplasia (BHMED) to this condition [5]. Some authors [1,13] argued that this family might be classified as a type of multiple epiphyseal dysplasia (MED). Others [12] classified it as a form of osteopetrosis with precocious manifestations. We found the clinical picture in this family and especially the radiological features consistently different from MED and to be unique in its presentation. The onset of complaints in this family was at about four years of age as compared to at about ten years in MED. In both disorders, complaints start in early childhood and stabilise during adolescence. In adulthood, complaints in BHMED worsen due to early-onset osteoarthritis. In the Elsbach family, mainly the hip and knee joint were bilaterally affected and harmful, whereas in MED also the elbow and ankle are involved [15]. Affected BHMED-individuals had a small total body height, but with an apparently proportionate build. The gait was waddling. The affected individuals had a smaller total body height than unaffected first-degree relatives. In turn, these unaffected relatives showed a smaller total body height than controls. In addition, the total body height of spouses of affected subjects was remarkably reduced compared to the control group and the reference population. Thus, this indicates that the short stature of offspring of affected patients can not only be attributed to the putative dominant mutation causing BHMED, but might also be the result of (epi-)genetic factors that play a role through height-related partner selection [9,16]. Furthermore, it shows that total body height is not a distinctive inclusion criterion when defining the affection status in a skeletal dysplasia with mild to moderate effect on final adult height.

Both BHMED and MED show an autosomal dominant mode of inheritance with a gender ratio of about 1. Radiographically, in BHMED the epiphyses of the proximal and distal femur and proximal (and distal) tibia appear rather late in comparison with their peers with subsequent flattening in the following years. Initially, during childhood, the epiphyses of the knee are irregular. In adolescence, the knee epiphyses show a more continuous aspect without irregularities. Only the joint surface is flattened, especially at the lateral condyle. Fairbanks described MED in 1935 [7] and gave a further delineation in 1947 [6]. In some cases the disease was familial. Short hands with brachydactyly, characteristic for MED [11,15] were not reported in BHMED. In the family described here, we found shortening of MC IV and V. In children with MED, a deficiency of the lateral part of the distal ossification centre of the tibia is observed, evolving into in a sloping end of the tibia at adulthood [11]. In contrast, in BHMED the distal tibia epiphysis is only small in size.

Other conditions with partial resemblance of epiphyseal ossification disturbances are mucopolysaccharidosis IV (M. Morquio-Brailsford, MPS-IV) and disorders characterised by punctuated chondrodysplasia. In BHMED some stippling on the periphery of the epiphyses (a sign of multiple ossification centres) may be observed, but never as prominent as in chondrodysplasia punctata [14]. In MPS-IV, epiphyseal dystrophy is found with typical changes in the spinal column and in most centres of ossification, especially of the hip. In contrast with BHMED, the acetabula are enlarged [10]. Furthermore, the mode of inheritance for both chondrodysplasia puncatata and MPS-IV is autosomal recessive, which clearly differentiates these conditions from BHMED.

The patient with radiological signs of a bilateral epiphysiolysis of the hip (III-5), was a coincidental finding without signs of BHMED.

The clinical features in this family with BHMED represent a consistent and distinct phenotype with multiple differences in comparison with MED. Therefore, BHMED possibly represents a specific skeletal dysplasia due to a unique genetic defect.

Future linkage analysis and characterisation of the molecular defect causing this disorder will clarify whether it is indeed allelic to other skeletal dysplasias like MED, or due to a mutation of a novel gene. This study demonstrates that BHMED as a distinct entity to be separated from classical MED. A dysfunction of the epiphyseal chondrocyte with subsequent delayed and irregular ossification might be the pathogenic basis of BHMED. Epiphyseal irregularity, together with the morphoplastic influence of both articular surfaces probably counts for the dyscongruence and secondary osteoarthritis.

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REFERENCES

1. Bailey JA. Disproportionate short stature. Differential diagnosis and management. First ed. Philadelphia: W.B. Saunders Co., 1973;429
2. Central institution for statistics (CBS) Vademecum gezondheidsstatistiek. Aspects of health and disease in the population. Edition 1998.Voorburg: Holland, 1998;302–317
3. Diamond LS. A family study of spondylo-epiphyseal dysplasia. J Bone Joint Surg [Br] 1970;52-B:1587–1594
4. Doege TC, Houston TP. American Medical Association: Guides to the Evaluation of Permanent Impairment. Fourth Edn. Chicago: American Medical Association, 1993
5. Elsbach L. Bilateral hereditary micro-epiphysial dysplasia of the hips. J Bone Joint Surg [Br] 1959 ;41-B:514–523
6. Fairbank HAT. Dysplasia epiphysealis multiplex. Br J Surg 1947;34:225–232
7. Fairbank HAT. Generalized disease of the skeleton. Proc R Soc Med (Clinical Section) 1935;28:1611–1619
8. Hegenbarth R. Special factors governing x-ray examination of the hip joint in children. Roentgenblatter 1980;33:18–30
9. Hensley WE. Height as a basis for interpersonal attraction. Adolescence 1994;29:469–474
10. Langer LO jr, Carey LS. The roentgenographic features of the KS mucopoly-saccharidosis of Morquio (Morquio-Brailsford's disease). Am J Roentgenol Radium Ther Nucl Med 1966;97:1–20
11. Leeds NE. Epiphyseal dysplasia multiplex. Am J Roentgen 1960;84:506–510
12. McKusick VA, Scott CI. A nomenclature for constitutional disorders of bone. J Bone Joint Surg [Am] 1971;53-A:978–986
13. McKusick VA. Mendelian Inheritance in man. A catalog of human genes and genetic disorders. Vol. 2, Eleventh Edn. Baltimore: Johns Hopkins Co., 1992;484–485

14. Motley AB, Tabak HF, Smeitink JA, Poll-The BT, Barth PG, Wanders RJ. Non-rhizomelic and rhizomelic chondrodysplasia punctata within a single complementation group. Acta Biochim Biophys 1996;1315:153–158
15. Mourik van JBA, Schaap C, Nollen AJG. Een Nederlandse familie met erfelijke gewrichtsklachten; Multipele Epiphysaire Dysplasie. Ned Tijdschr Geneeskd 1993;137:32–36
16. Roscoe B, Diana MS, Brooks RH. Early, middle and late adolecents' views on dating and factors influencing partner selection. Adolescence 1987;22:59–68
17. Stevens PM, Coleman SS. Coxa breva: Its pathogenesis and a rationale for its management. J Pediatr Orthop 1985;5:515–521

4.2. RADIOLOGICAL FEATURES OF BILATERAL HEREDITARY MICRO-EPIPHYSEAL DYSPLASIA

– A distinct entity in the skeletal dysplasias –

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RöFo Fortschr Geb Röntgenstr Neuen Bildgeb Verfahr 2002;174(7):887–892.

ABSTRACT

Aim of the study: to prove that bilateral hereditary micro-epiphyseal dysplasia (BHMED), first described by Elsbach in 1959 [1], is a distinct disorder radiologically as well as clinically, compared with multiple epiphyseal dysplasia (MED).

Materials and methods: we used the data of the revised pedigree with 84 family members, performed a medical history, physical examination and made a radiological evaluation for making a clinical and radiological phenotype of BHMED family members. We used blood samples for genetic analysis.

Results: although there is a clear clinical picture of the dysplasia, the radiological signs are more reliable at making the diagnosis. Especially the typical deformity of the hip and knee joint are diagnostic for BHMED. By linkage analysis we excluded linkage with the three known MED-loci (EDM1, EDM2 and EDM3).

Conclusion: BHMED is indeed an entity that is distinct from common multiple epiphyseal dysplasia (MED), clinically, as well as radiologically as well as genetically.

INTRODUCTION

Bilateral hereditary micro-epiphyseal dysplasia (BHMED) is a rare skeletal dysplasia, in which predominantly the hips and knees are affected. In 1959, Elsbach gave the first description of an unique Dutch family with this disorder [1]. The radiological observation of small epiphyses in hip, knee and shoulder urged him to designate the disorder as BHMED. The affected joints are involved simultaneously and bilaterally. The pedigree of this family was revised and

updated. This enabled us to further delineate the clinical and especially the radiological phenotype.

To establish that BHMED is indeed a clinical and radiological other dysplasia than the well known MED, we performed a linkage analysis. For multiple epiphyseal dysplasia (MED) three genetic loci (EDM1, EDM2 and EDM3) have been identified on chromosomes 19p13.1, 1p33-p32.2 and 20q13.3, respectively. EDM1 is associated with mutations in the cartilage oligometric matrix protein (COMP), EDM2 is associated with mutation in the alpha-2 chain of type IX collagen (COL9A2) and EDM3 is associated with mutations in the alpha-3 chain type IX collagen (COL9A3). No evidence for linkage of BHMED in this family was found to any of the three currently known MED loci.

MATERIAL AND METHODS

After informed consent, from each family member a medical history was taken according to a standardised protocol. The family exists of 84 members in six generations of which 44 were clinically relevant. Physical examination was done according to the guidelines for the Evaluation of Permanent Impairment (4th edition) of the American Medical Association [2].

For the clinical diagnosis of BHMED, we used the clinical description according to Elsbach [1], i.e., bilateral simultaneous onset of complaints of pain in hip and knee joints, an apparently short stature, and in addition waddling gait and restricted range of motion of the hip. Persons younger than four years of age were excluded from this study, since onset of complaints was rarely if ever before this age. Patients and relatives with these clinical symptoms were categorised as affected. Persons who did not fulfil these clinical symptoms, or who were not

examined or only incompletely examined were categorised as inconclusive with respect to affection status. Only patients who underwent a complete examination and showed no clinical signs of BHMED were categorised as clinically unaffected. Roentgenographs made in the past were retrieved and supplemented with new ones when clinically indicated. For radiological description of the state of affectedness, all roentgenographs were reviewed by a radiologist specialised in skeletal dysplasias (P.F.D.), without prior knowledge of the clinical and pedigree information.

We also measured the width of both condyles (X) and the height of the intercondylar notch (Y) on the plain antero-posterior (AP) film. In a standardised way and only with a fully extended lower extremity, an X/Y ratio was computed as an indirect but quantifiable measure of the severity of the dysplasia, as shown in figure 2.

In affected family members, routine laboratory examination was done. Blood samples were taken for DNA extraction.

Polymorphic markers flanking the COMP gene (D19S226 and D19S414), the COL9A2 gene (D1S255 and D1S2797) and the COL9A3 gene (D20S196, D20S100 and D20S173) were used to test for linkage to any of these three loci. We used a highly conservative genetic model for our linkage analysis; BHMED was assumed to be an autosomal dominant disorder with a gene frequency of 1:1000 with full penetrance.

DNA was available from 22 family members: 12 affected family members (III-2, III-23, III-27, IV-3, IV-15, IV-18, IV-21, IV-28, V-1, V-4, V-5 and V-6), eight unaffected family members (III-5, III-8, III-9, III-29, IV-19, IV-23, IV-25 and V-13) and of two healthy spouses who had affected offspring (III-22 and IV-14), according to the pedigree (Fig. 1). Family members who were radiologically affected (II-1 and II-6) or affected according to Elsbach as indicated in the current

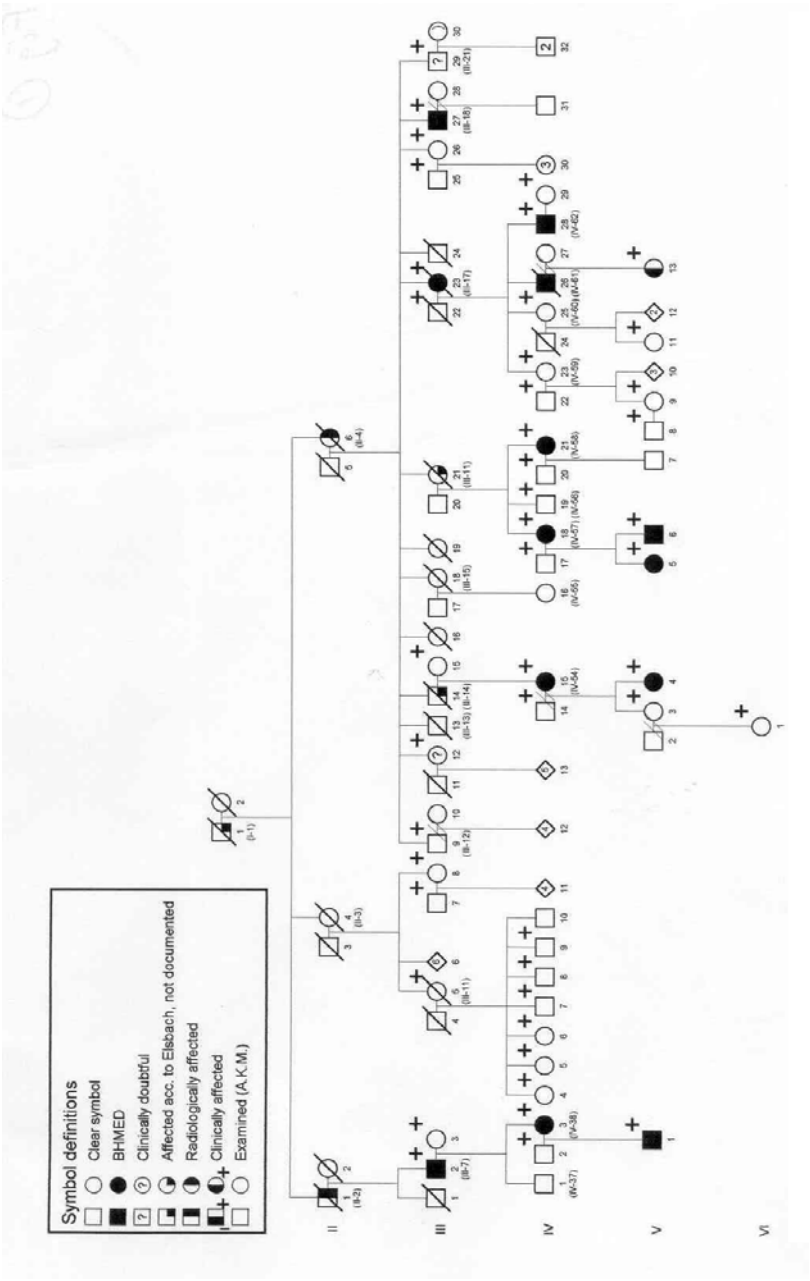

Figure 1. Pedigree of the family. The black symbols characterise affected family members; white symbols are for the unaffected individuals.

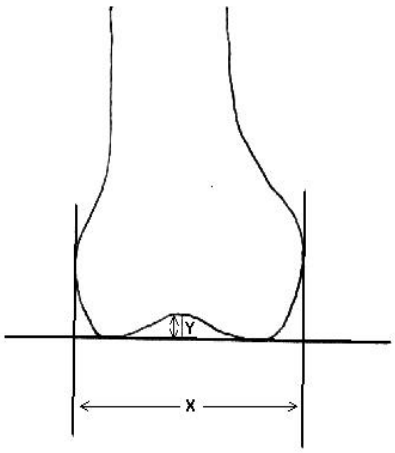

Figure 2. Measurement of the dysplasia of the knee by the X/Y ratio of the width of the condyles (X) relative to the height of the intercondylar notch (Y).

pedigree (I-1, III-14 and III-21) (Fig. 1) were also classified as affected in the linkage analysis.

RESULTS

Pedigree. The current pedigree (Fig.1) encompasses 6 generations and shows an apparently autosomal dominant mode of inheritance with a male/female gender ratio of affected persons close to unity. All subjects who had an affected parent and at least one affected child, showed clinical and radiological symptoms of BHMED. This suggests that at adult age the penetrance of the gene defect is high. In comparison with the pedigree published by Elsbach [1], the pedigree is extended with subsequent generations. In addition, one branch of the family appeared to be clinically and radiologically unaffected whereas Elsbach supposed them to be affected.

Physical examination. In general, the clinical picture of an affected BHMED family member showed a short stature and a waddling gate with limping. There was a loss of hip function, especially in the abduction (limited with an average of 20 degrees) and in the internal rotation (limited with an average of 25 degrees). Only above the age of 60, there were flexion-contractures of the knee. The shoulders showed no limitation of function, even at older age, although they were painful in movements above the age of 50 in the affected. The hands appeared to be relatively broad and short. When clenching the fists, a shortening of the metacarpals (MC), especially MC IV and V was noticed. In some subjects, the feet showed a slight proximal syndactyly at the second webspace.

Figure 3. Pelvis of a 45-year-old woman with BHMED, showing a valgus angle of the femoral neck which is short and thickened, flattening of the head of the femur and the femoral epiphysis. Remark the interesting teardrop configuration in the acetabulum.

Table I. Summary of radiological observations (N of abnormal dysplastic joints / N of radiologically evaluated joints of affected and unaffected subjects).

N = 28	Shoulders	Elbows	Wrists	Spine	Hips	Knees	Ankles
Small epiphyses	6/12	1/1			15/28		2/5
Small epicondyles		1/1				15/28	
Ulna plus			11/15				
Spondylosis				7/7			
Flattened head					15/28		
Short valgus neck					15/28		
Shallow notch						15/28	

Despite the high rate of complaints of low back pain, physical examination showed no abnormalities, except for lumbosacral fixation in some subjects.

Radiological examination: Radiographic evaluation was possible in twenty-eight family members (Table I).

Hip abnormalities (Fig. 3) were characterised by a valgus angle of the femoral neck [3] which is short and thickened [4], flattening of the head of the femur and the femoral epiphysis especially at the medial part – at the site of the fovea centralis – , and dysplasia of the acetabulum as expressed by a lack of development of the normal teardrop configuration of the medial acetabular wall.

The abnormalities of the knee were characterised by dysplastic deformities like flattened and small condyles (Fig. 4a,b), especially on the lateral view in contrast with unaffected family members. In a few cases there was a focus of osteo-chondritis dissecans and in others an ulcus was seen at the lateral femoral condyle.

Table II. The intercondylar notch X/Y ratio.

N = 28	Notch X/Y ratio (range)
Affected (n = 15)	12.17 (9.29 – 15.13)
Unaffected (n = 13)	9.75 (7.15 – 12.10)

Figure 4. (**a**) AP-view of the left knee of a 42-year-old man with BHMED, showing the flattened and small condyles, and the shallow intercondylar notch. (**b**) Lateral view of the same left knee (a), showing the small epiphyses and small condyles. (**c**). MRI of the same left knee (b), showing an irregular thickness of the cartilage cover. (conventional gradient echo-pulse sequence, $T2^{*}$ weighting, small flip angle 5˚–20˚, long TE 15–25 ms, short TR).

All affected family members showed a shallow intercondylar notch on the AP view of the knee compared to the unaffected family-members. As shown in figure 2, the X-value represents the width of both condyles and the Y-value represents the height of the intercondylar notch. For affected and unaffected family members, the ratio between the width of the condyles (X) and height of the intercondylar notch (Y) is given in Table II.

The facies patellaris of the distal femur was not flattened on the axial patellar view.

Figure 5. PA-view of both hands (and wrists) of an affected 52-year-old woman, showing an 'ulna plus' with slight radial drift of the carpus and flattening of the ulnar part of the radial epiphysis. The hands show short metacarpals of the fourth and fifth digits, but also a shortening of the proximal and midphalanges. The distal phalanges showed normal lengths.

In some affected family members additional magnetic resonance imaging of the knees was performed, showing an irregular thickness of the cartilage cover (Fig. 4c).

In all affected family members an 'ulna plus' with slight radial drift of the carpus and flattening of the ulnar part of the radial epiphysis was observed. X-rays of the hands showed short metacarpals of the fourth and fifth digits, but also a shortening of the proximal and midphalanges. The distal phalanges showed normal lengths (Fig. 5).

The humeral head showed a small and flattened epiphysis and a shallow glenoid surface with premature osteoarthritis above the age of 50 (Fig. 6).

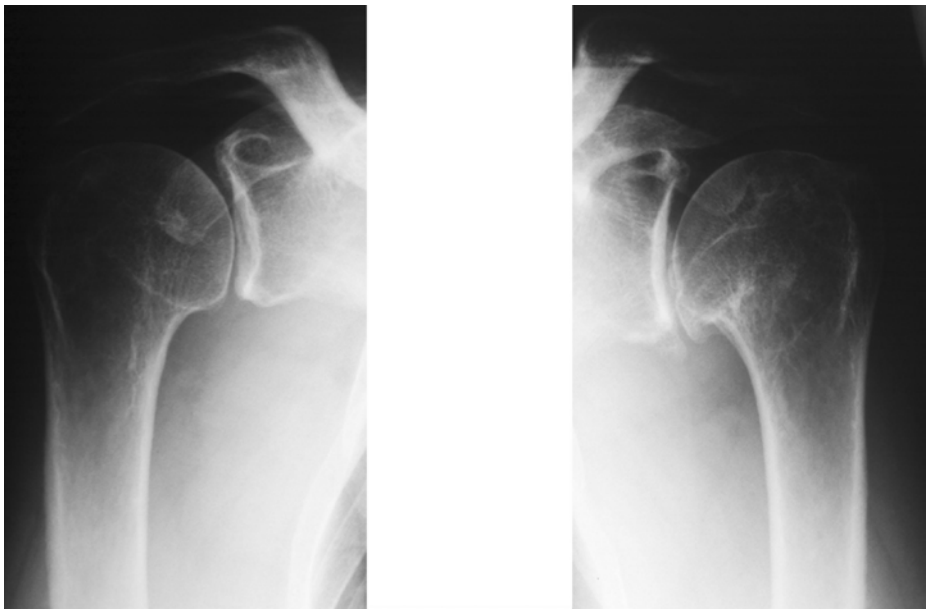

Figure 6. AP-view of both shoulders of a 73-year-old woman having BHMED, showing the small and flattened epiphysis and a shallow glenoid surface with premature osteoarthritis.

Radiologically, the spine showed only signs of lumbar spondylosis, whereas the cervico-thoracic spine was normal. Flattened vertebrae as occur in e.g. spondylo-epiphyseal dysplasia [5], were not observed.

From three clinically and radiologically affected persons, radiographs of the whole upper and lower extremity were available. Although there were no or minor complaints of elbow and ankle joints, the X-rays of the separate joints showed small epiphyses of distal humerus and distal tibia.

In summary, affected joints were characterized by small epiphyses without involvement of the metaphyses.

Laboratory examination. The routine laboratory examination in affected family members showed normal results, ruling out a number of metabolic bone disorders.

Table III. Two point LOD scores between BHMED and markers flanking the EDM1-3 loci.

	Recombination fractions (cM)						
Marker	0	0.01	0.05	0.10	0.20	0.30	0.40
D19S226	- ∞	-1.61	-0.93	-0.62	-0.30	-0.12	-0.03
D19S414	- ∞	-1.01	-0.41	-0.22	-0.07	-0.03	-0.05
D1S255	- ∞	-3.12	-1.49	-0.81	-0.28	-0.10	-0.03
D1S2797	- ∞	-5.73	-3.01	-1.90	-0.90	-0.41	-0.14
D20S196	- ∞	-9.27	-4.59	-2.73	-1.15	-0.47	-0.16
D20S100	- ∞	-7.01	-3.58	-2.16	-0.90	-0.33	-0.07
D20S173	- ∞	-7.90	-3.91	-2.34	-0.99	-0.39	-0.10

The currently known gene loci of EDM1-3 (D19, D1, D20) show very low LOD-scores, showing that BHMED can be excluded as being one of the EDM1-3 dysplasias.

Genetic examination. Table III summarises the results of linkage analysis showing no evidence for linkage of BHMED in this family to any of the three currently known MED loci (EDM1, EDM2 and EDM3). Most of the tested markers provided strong evidence for non-linkage.

DISCUSSION

At the moment, more than 190 skeletal dysplasias are known, divided in 32 groups [6].

We re-examined the family initially studied by Elsbach. We found strong clinical, radiological, and molecular genetic support for his assumption that the skeletal disorder in this family represents a unique clinical entity, that has to be distinguished from common MED. Radiological evaluation of the joints involved in BHMED showed a unique pattern of abnormalities. The morphogenesis of the joints is disturbed, as is seen in the flattened dysplastic femoral head with its small epiphyses and the likewise dysplastic acetabulum. The same pattern is seen in the knee joint, at the femoro-tibial site as well as at the patellar-femoral site, of which the shallow intercondylar notch is an additional sign. The facies patellaris of the distal femur seems to be less flattened, especially seen on the axial view.

Due to the deformed joint surface, early onset osteoarthritis seemed to be induced, as was observed in many family members.

There was only a general shortening of the long bones, due to affected proximal and distal epiphyses. Predominantly rhizomelic or mesomelic deformities, proximal focal femoral deficiency and other syndromes could be ruled out in this way.

The width of the condyles (X) relative to the height of the intercondylar notch (Y) showed indeed a clear difference between family members with BHMED and unaffected relatives (Table II). However, the X/Y ratio showed wide overlapping values for the two groups of affected and unaffected subjects and this limits its diagnostic value. Furthermore, this ratio does not reflect the irregularities of the cartilage as can be visualised with MRI (Fig. 4c).

Once Elsbach published his description of the disorder in this family, some authors [7,8] suggested that BHMED was in fact a variant of MED, and not a distinct clinical entity.

We re-examined this family to delineate further the clinical picture and to establish whether BHMED is a variant of or identical to MED or indeed, as suggested by Elsbach, a distinct clinical entity.

In the 'Elsbach family', the well-developed ossification centre of the epiphyses are small and homogeneous, whereas in MED accessory ossification centres are seen, scattered in the periphery of the cartilaginous epiphyses. The metacarpals and phalanges of affected relatives were short, but the distal phalanges were not stubby as in MED [9]. Although affected subjects showed deformity of the ulnar part of the distal radial epiphysis, the 'V' wrist joint deformity [10] due to hypoplasia of the adjacent portions of the radial and ulnar epiphyses in MED, was not observed in the currently studied family. In addition, the carpal bones were of normal size without irregularities, whereas they are small and irregular in MED.

In the 'Elsbach family', the spine is not involved, neither clinically nor radiologically. In MED, the spine shows minimal flattening and reduction of the vertebral bodies, but vertebral changes may be quite similar to Scheuermann's disease because of the intraspongious herniations of the lower thoracic or first lumbar vertebrae. With the appearance of the apophyses, the rim of the vertebral

body shows fragmentation in MED and, infrequently, a vertebral body will be fragmented [11].

Other signs like 'slant sign' of the ankle (thinning of the lateral part of the lower tibial epiphyses), 'flat-top' talus, and early onset osteoarthritis were non-specific for the distinction of different dysplasias. In particular, these features do not provide further distinction between these conditions, because they were observed at low frequency in both, or because of low denominator for each type of joints studied.

In the 'Elsbach family' the femoral head is medially flattened in each affected relative, whereas in MED a large variation of abnormalities may be observed ranging from normal to gross deformation [12].

With linkage analysis, the three currently known loci for MED (EDM1, EDM2 and EDM3) were excluded as candidate genes for BHMED. This supports that BHMED represents an unique entity which is distinct from common MED. Future linkage analysis and mutation analysis of positional and functional candidate genes should reveal whether BHMED is allelic to another skeletal dysplasia or due to a mutation in a novel gene.

CONCLUSION

Bilateral hereditary micro-epiphyseal dysplasia (BHMED) is a separate entity in the list of the epiphyseal skeletal dysplasias. Although there is a clear clinical picture of the dysplasia, the distinctive radiological signs are more reliable for making the diagnosis in new patients. Especially the typical deformity of the hip and knee joint, that appear symmetrically and simultaneously, are diagnostic for having BHMED. By linkage analysis we could exclude that BHMED is a form or variant of common multiple epiphyseal dysplasia (MED).

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REFERENCES

1 Elsbach L. Bilateral hereditary micro-epiphysial dysplasia of the hips. J Bone Joint Surg 1959;41B:514–523.

2 Doege TC, Houston TP. American Medical Association: Guides to the Evaluation of Permanent Impairment. Fourth ed. Chicago: American Medical Association, 1993.

3 Hegenbarth R. Special factors governing x-ray examination of the hip joint in children. Roentgenblatter 1980;33:18–30.

4 Stevens PM, Coleman SS. Coxa breva: Its pathogenesis and a rationale for its management. J Pediatr Orthop 1985;5:515–521.

5 Diamond LS. A family study of spondyloepiphyseal dysplasia. J Bone Joint Surg 1970;52B:1587–1594.

6 Rimoin DL. International Nomenclature and Classification of the Osteochondrodysplasias (1997). International Working Group on Constitutional Diseases of Bone. J Med Gen 1998;79:376–382.

7 McKusick VA. Mendelian Inheritance in man. A catalog of human genes and genetic disorders, Vol. 2, Eleventh edn. Baltimore: Johns Hopkins Co., 1992;484–485.

8 Bailey JA. Disproportionate short stature. Differential diagnosis and management. First edn. Philadelphia: W.B. Saunders Co., 1973;429.

9 Mourik van JBA. Multiple epiphyseal dysplasia. A clinical and molecular genetic study. Thesis, Rotterdam, 1998.

10 Hoefnagel D, Sycamore LK, Russell SW, Bucknall WE. Hereditary multiple epiphyseal dysplasia. Ann Hum Genet 1967;30:201–210.

11 Rubin P. Dynamic Classification of Bone Dysplasias. Chicago, Year Book Medical Publishers Inc., 1964;146–159.

12 Barrie H, Carter C, Jutcliffe J. Multiple epiphyseal dysplasia. Br Med J 1958;2:133–137.

4.3. FAMILIAL MULTIPLE EPIPHYSEAL DYSPLASIA DUE TO A MATRILIN-3 MUTATION

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American Journal of Medical Genetics 2003; in press

[†]Shortly after submission of this manuscript, Piet Dijkstra died unexpectedly. May this article be dedicated to him and memorize his extensive contributions to genetic studies of skeletal dysplasias.

ABSTRACT

In this study, we followed-up the family with bilateral hereditary micro-epiphyseal dysplasia (BHMED) originally described by Elsbach in 1959. Clinical re-examination of all available family members resulted in further delineation of the clinical and radiological phenotype, which is distinct from common multiple epiphyseal dysplasia (MED). Linkage analysis excluded EDM1, EDM2 and EDM3 as candidate genes. Linkage and mutation analysis of matrilin-3 revealed a new pathogenic mutation confirming that BHMED is indeed a distinct disease entity among MED and MED-like disorders.

INTRODUCTION

Bilateral hereditary micro-epiphyseal dysplasia (BHMED) is a rare, usually symmetrical skeletal dysplasia first reported by Elsbach [1959]. In the family he described, several members complained mainly of pain in hips and knees from early childhood. Because of the radiological finding of small epiphyses in the hip and knee joint, Elsbach designated the disorder bilateral hereditary micro-epiphyseal dysplasia. BHMED should be differentiated from multiple epiphyseal dysplasia (MED). Recently, several members of the original family were referred to the Department of Orthopaedic Surgery in Delft with questions about the genetic background, and the recurrence risk. We re-examined the extended family, in order to evaluate diagnostic criteria and the clinical course, to determine the clinical spectrum, and to provide tools for the diagnosis of possibly affected persons with only partial expression of the disease.

By mutation analysis we identified a pathogenic mutation within the matrilin-3 gene in our family.

This study illustrates the value of detailed and accurate description of the phenotype as a basis for clinical follow-up studies and future genetic studies, and further delineates the clinical spectrum of matrilin-3 related disorders.

MATERIALS AND METHODS

The pedigree was updated and contained 54 subjects in six generations. Thirty-two members of the family and 12 spouses were examined by the first author (A.K.M.). After informed consent, all family members agreed to full cooperation with the study. This study was approved by the Medical Ethics Committee of the Reinier de Graaf Hospital in Delft, the Netherlands.

A full medical history was taken according to a standardized questionnaire. Physical examination was performed according to a standard protocol. The range of motion of hip, knee, and shoulder joints was measured according to guidelines (4th edition) of the American Medical Association (Doege et al., 1993). Total body height, sitting height, leg length, and span width were measured and compared with reference data from the Dutch population (CBS, 1998).

Prior and additional radiographs of first- and second-degree family members with complaints were evaluated by one of the authors (P.F.D.). In some of the cases, results of laboratory tests of serum calcium, phosphate and alkaline phosphatase levels were available and collected.

Diagnostic criteria. All subjects over 4 years old were classified clinically and radiologically as affected, unaffected, or inconclusive (included not known).

Clinical classification. The original clinical description by Elsbach [1959] was used as the basis for the clinical diagnosis of BHMED. Diagnostic criteria were bilateral simultaneous onset of pain in hip and knee joints, short stature, waddling gait and restricted range of motion of the hip. Patients and relatives who fulfilled all clinical diagnostic criteria were categorised as affected (group I). Subjects who did not fulfil all diagnostic criteria or who were not examined or only incompletely examined were categorized as inconclusive with respect to clinical affection status (group II). Patients who showed no clinical signs of BHMED were categorized as unaffected (group III).

Radiological classification. All radiographs were reviewed by a radiologist specialized in skeletal dysplasias (P.F.D.), without knowledge of the clinical evaluation or the *a priori* risk for the condition. The diagnostic criteria for radiological status were (a) a hip characterized by valgus angle of the femoral neck (Hegenbarth, 1980) which is short and thickened (Stevens et al., 1985); (b) flattening of the head of the femur especially at the medial part, dysplasia of the acetabulum by an apparently abnormal teardrop configuration of the medial acetabular wall (Fig. 2); (c) small epiphyses of proximal and distal femur and proximal tibia with or without flattened condyles of the knees and a shallow intercondylar notch (Fig. 3); and (d) symmetrical distribution of abnormalities. Subjects fulfilling the first, second, and fourth criteria (minimal diagnostic criteria) were regarded as radiologically affected. Patients not fulfilling these criteria were classified as radiologically unaffected.

One of the two index patients in the publication by Elsbach [1959] died before re-examination (Fig. 1, IV-26). This was the only patient we regarded as affected solely on the basis of the clinical and radiological description by Elsbach. The updated case history of the second index patient (Fig. 1, IV-28) is described in the results.

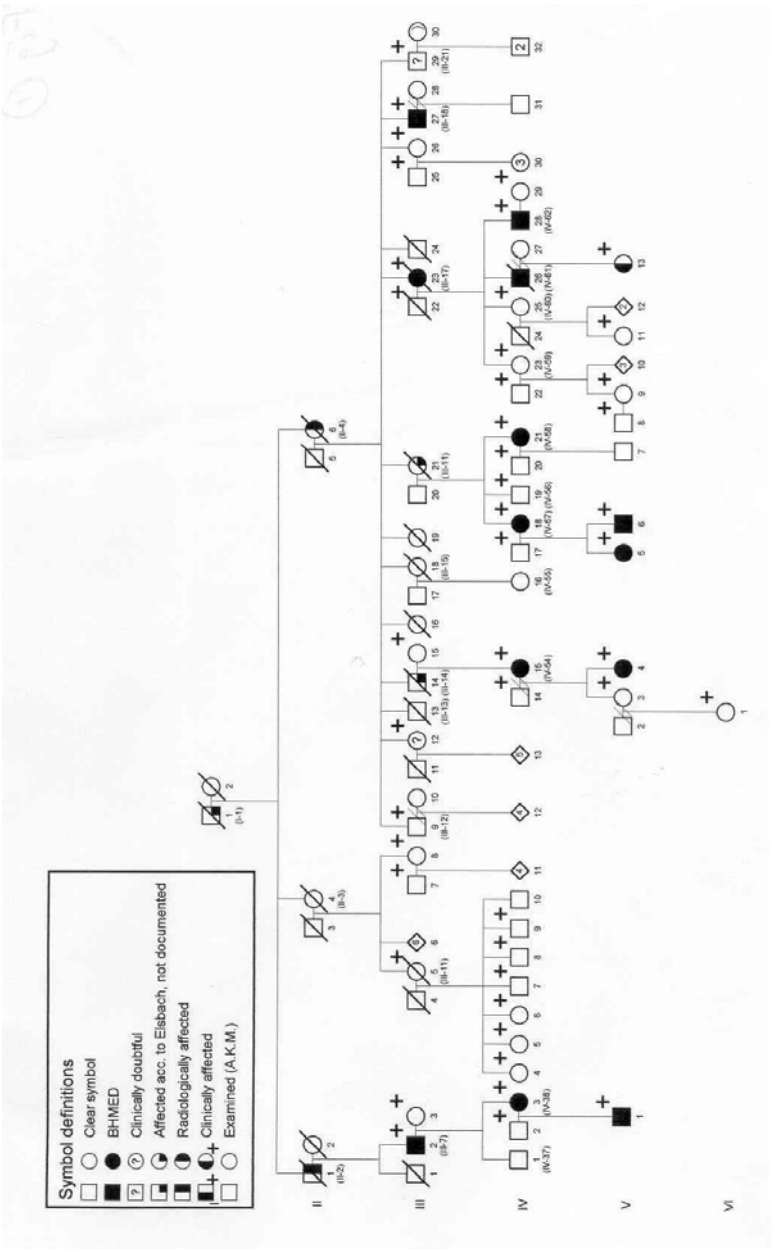

Figure 1. Revised pedigree of the BHMED family. Identification numbers between brackets represent identification numbers in the pedigree originally published by Elsbach [1959].

Figure 2. AP-radiograph of the pelvis and hips of a BHMED patient showing flattening of the femoral head, a short and thickened femoral neck and an abnormal teardrop configuration of the medial acetabular wall.

GENETIC ANALYSIS

Genetic studies. Genomic DNA was isolated from peripheral blood as described (Miller et al., 1988). DNA was available from 12 affected family members (III-2, III-23, III-27, IV-3, IV-15, IV-18, IV-21, IV-28, V-1, V-4, V-5 and V-6), eight unaffected family members (III-5, III-8, III-9, III-29, IV-19, IV-23, IV-25 and V-13) and of two healthy spouses who had affected offspring (III-22 and IV-14) (Fig. 1). Family members who were radiologically affected (II-1, II-6) or affected according to Elsbach as indicated in the current pedigree (I-1, III-14, III-21) (Fig. 1) were also classified as affected in the linkage analysis. Two point linkage

analysis was done using the MLINK program of the LINKAGE package (version 5.1) (Lathrop et al., 1984). LOD and location scores were calculated assuming BHMED to be an autosomal dominant disorder with 100% penetrance and a gene frequency of 1:10,000. No phenocopies were allowed and allele frequencies were calculated from the family. For multiple epiphyseal dysplasia, three genes (EDM1-3) have been identified on chromosomes 19p13.1, 1p33-p32.2 and 20q13.3, respectively. EDM1 is associated with mutations in the cartilage oligometric matrix protein (COMP), EDM2 is associated with mutation in the alpha-2 chain of type IX Collagen (COL9A2) and EDM3 is associated with mutations in the alpha-3 chain type IX Collagen (COL9A3). Short tandem repeat polymorphisms (STRPs) flanking the COMP gene (D19S226 and D19S414), the COL9A2 gene (D1S255 and D1S2797) and the COL9A3 gene (D20S196, D20S100 and D20S173) were used to test for linkage to any of these three loci.

For the systematic genome scan STRPs from the Marshfield screening set 6A (http://research.marshfieldclinic.org/genetics/) were amplified using 25 ng genomic DNA in 10 μl PCR reactions containing 1X GeneAmp PCR Gold Buffer; 1.5 mM $MgCl_2$; 25 ng of fluorescent forward primer; 25 ng of unlabelled reverse primer and 0.4 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). Initial denaturation was 10 min at 94^{o}C followed by 32 cycles of 30 s denaturation at 94^{o}C, annealing at 55^{o}C and 90 s extension at 72^{o}C. PCR products were loaded on an ABI3100 automated sequencer (filterset D), data were analyzed using ABI GeneMapper 1.0 software. The genomic structure of *matrilin-3* (*MATN3*) was determined by aligning cDNA (NM_002381) with genomic sequence NT_005214. Primers were designed to amplify DNA from coding regions, splice sites and at least 50 bases of flanking intronic sequence on both sides of each exon. The used primers are listed in Table I.

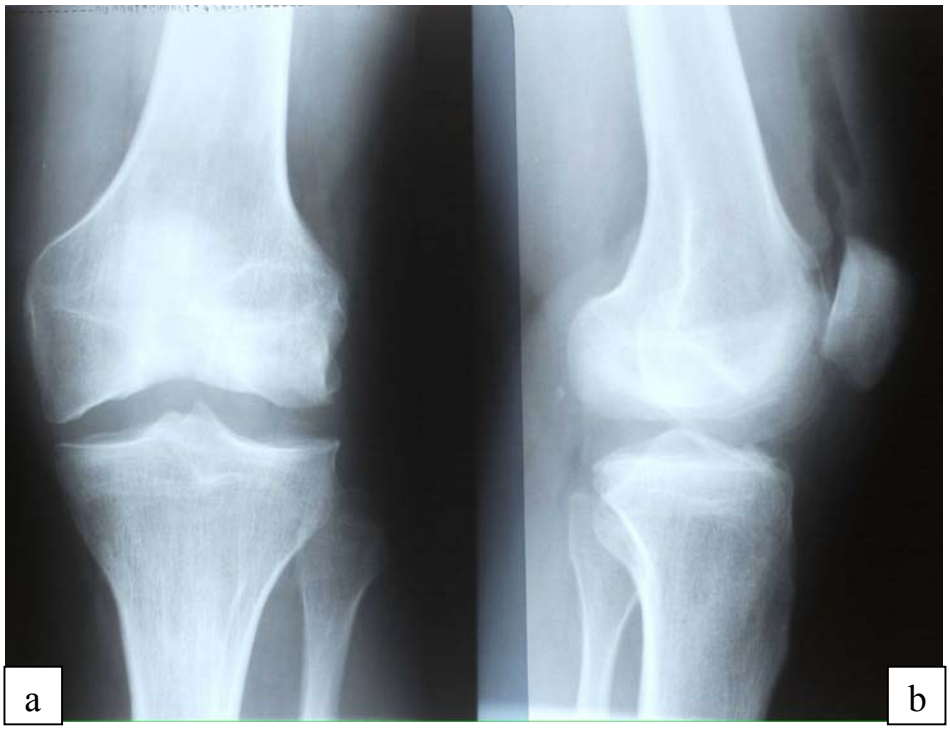

Figure 3. Radiographs of the left knee of a BHMED patient (Fig. 3a, AP-view; Fig. 3b, lateral view) showing small epiphyses, a shallow intercondylar notch due to flattened condyles and osteo-arthritic changes.

Table I. Used primers to amplify DNA from coding regions, splice sites.

Exon	Forward	Reverse	Size (bp)
1	cgcggacaaggctccttg	ctcctcgttcgatgctcac	430
2_1	tcctggaatcccagagttc	ctcattcacctggtcctg	472
2_2	tggacgaagccttcacagtg	ctctgggtacacaatgtctg	347
3	cagagagcaatgtctattga	tacaaagtatggaaataagctg	424
4	ataactgtaagtggcgtggc	tcccagtcaatgtgacctgc	293
5	tttcctcatatgtttccagc	gagcaatgtgtgccttcatc	371
6	gactactacattctcagcac	cacaactgtgtcttagacag	331
7	cacttaggtaagtcttaacg	tcacttgcctgtggaatgtc	316
8	ttcacttctcataggacata	gaatcatgctgattctgcag	339

PCR reactions for exon 1 were performed in 25 µl containing 10 X Cloned PFU PCR buffer (Stratagene); 200 µM dNTP; 1 µM forward primer; 1 µM reverse primer; 1.25 units Cloned PFU polymerase (Stratagene, La Jolla, CA, USA) and 50 ng genomic DNA. Cycle conditions: 10 min at 98°C; 11 cycles of 30 s denaturation at 98°C, 30 s annealing at 68–1°C per cycle, and 2 min extension at 75°C; followed by 24 cycles of 30 s denaturation at 98°C, 30 s denaturation at 58°C and 2 min extention at 75°C; final extension 5 min at 75°C. Exon 2_1 and exon 2_2 were amplified in 50 µl containing 10X PCR Gold buffer (Perkin Elmer Life Sciences Inc., Boston, MA, USA); 1.0 mM $MgCl_2$; 200 µM dNTP; 0.8 µM forward primer; 0.8 µM reverse primer; 2.5 unit Ampli Taq Gold polymerase (Perkin Elmer Life Sciences Inc.) and 50 ng genomic DNA. Exon 3, 4, 5, 6, 7 and 8 were amplified in 50 µl containing 10X PCR Gold buffer (Perkin Elmer Life Sciences Inc.); 1.5 mM $MgCl_2$; 200 µM dNTP; 0.8 µM forward primer; 0.8 µM reverse primer; 2.5 unit Ampli Taq Gold polymerase (Perkin Elmer Life Sciences Inc.) and 50 ng genomic DNA. Amplification was done using 7 min initial denaturation at 94°C; 35 cycles 30 s at 94°C, annealing 30 s at 55°C (for exon 2_1), at 58°C (for exon 2_2, 4, 5, 6 and 7) or at 50°C (for exon 3 and 8), 1 min 30 s extension at 72°C; final extension 5 min at 72°C. PCR products were purified using the Millipore Multiscreen PCR plates, and their approximate concentration was determined using Low DNA Mass Ladder (GibcoBRL, Lifetechnologies, Rockville, USA). After direct sequencing of both strands using BigDye Terminator chemistry Version 3 (Applied Biosystems); Millipore Multiscreen-HV plates containing Sephadex G50 (Amersham Biosciences AB, Uppsala, Sweden) were used to purify the sequenced PCR products. Products were loaded on an ABI3100 automated sequencer and analysis was done with SeqScape Version 1.0 for heterozygous base calls and sequence alignment.

Testing of the base changes/deletion in exon 2_1 in the family and 200 control chromosomes was done using Allele Specific Oligo Hybridization (ASO). PCR products containing exon 2_1 were blotted onto Hybond-N+ (Amersham Biosciences). The blots were hybridized at 1 hour at 40°C in 5X SSPE; 1%SDS and 0.05 mg/ml single strand salmon sperm DNA with either the normal (gaactatgctagcac) or mutated (gaactatcctagcac) sequence primer.

Filters were washed until a final stringency of 0.3X SSC/0.1% SDS at 40°C.

RESULTS

Case history. Patient IV-28 (Fig. 1) was examined by Elsbach [1959] when he was five years of age. At that age, he had a history of hip and knee complaints for about one year, consisting of a limping and waddling gait, and difficulties in running and climbing stairs. Abduction and internal rotation were reported to be limited. At the age of 28, a total hip replacement was performed on the left side, which was revised 3 and 4 years later. Despite an acetabulum enlargement on the right side at 16 years, osteoarthritis developed and at 37 years that hip had to be replaced as well. Two years later an open Neer-plasty of his non-dominant left shoulder was performed because of complaints of impingement, although no radiological signs of shoulder dysplasia existed. At the age of 34 years, arthroscopy showed mild degeneration of the cartilage of the right knee. Nine years later a total knee replacement followed on that side. Although this subject was the smallest in height among his peers during primary school, he eventually reached a total body height (TBH) of 1.71 m. (<15th centile for the reference population [CBS, 1998]).

Table II. Summary of radiological versus clinical affection status.

Radiological affection status (Group)	Clinical affection status				
	Affected (I)	Inconclusive** (II)		Unaffected (III)	Total
		Doubtful	Unknown		
Affected	13	–	2*	–	15
Inconclusive**					
– Doubtful	–	1	–	–	1
– Unknown	–	1	–	1	2
Unaffected	1	–	–	9	10
Total	14	2	2	10	28

* This table includes two patients with radiographs previously published by Elsbach [1959] but without clinical re-examination in the current study.
** Inconclusive is split up into doubtful and not known (see section methods/diagnostic criteria).

Pedigree. The updated pedigree (Fig. 1) shows only those subjects of whom information about the phenotype was available. Elsbach (1959) reported one branch of this family to be 'certainly affected', without any clinical description. However, after re-examination of subject III-5, her offspring, and III-8, these relatives were classified as clinically and radiologically *un*affected with BHMED.

In 6 successive generations, 15 subjects were clinically and/or radiologically affected, and the male/female ratio was close to 1. This pattern of familial occurrence and possibly one occasion of male-to-male transmission (II-1→III-2) suggest an autosomal dominant mode of inheritance. All subjects with an affected parent and at least one affected child, and who had been examined clinically and radiologically, showed clinical expression, suggesting that at adult age the penetrance of the gene defect is high.

Clinical findings. Six men – five according to the clinical criteria, and one on the basis of Elsbach's description [1959] – and eight women were classified as clinically affected (group I); for one male and one female at 50% prior risk, the

clinical status was inconclusive (group II), whereas in two men and eight women at 50% prior risk, the diagnosis of BHMED was clinically excluded (group III) (Table II).

The clinically affected subjects (group I) clearly showed reduced femur abduction and internal rotation compared to group III (unaffected), whereas group II (inconclusive) is too small and heterogeneous for meaningful comparison.

Radiological findings. The six men and eight women who were classified as affected according to clinical criteria (group I) were also classified as affected according to radiological criteria, except one (Fig. 1, V-13). This woman fulfilled the clinical diagnostic criteria, but the radiographs were considered normal (Table II, lower left cell). The remaining three men and nine women were radiologically classified as unaffected or inconclusive. Thus, none of the evaluated subjects were classified as clinically unaffected and radiologically affected. Table III summarizes the clinical and radiographic findings of affected persons, as well as the age and the frequency of surgical interventions.

Routine laboratory data. Available results from additional laboratory measurements including calcium, phosphate and alkaline phosphatase levels in serum were within the normal ranges, according to the references of the laboratories and hospitals involved.

Genetic analysis. Through linkage analysis the three currently known loci for MED, EDM1-3, could be excluded as candidate genes for BHMED (Table IV).

In the systematic genome scan we obtained mildly positive LOD scores for markers on chromosome 2. Testing additional markers in this region (Table V) confirmed these findings and two-point linkage analysis yielded a maximum LOD

Table III. Clinical and radiographic findings and surgical interventions for all affected individuals.

	N (results/all affecteds)
Clinical and radiological symptoms	
Limping/waddling gait	15/15
Limited hip-joint function	15/15
Early onset arthritis hip	15/15
Short MC IV & V	15/15
Small/irregular epiphyses	15/15
Coxa valga	15/15
Short thick femoral neck	15/15
Flattened femoral head	15/15
Shallow notch knee	15/15
Surgical procedures	
Total hip replacement (THR)	9/15
Total knee replacement (TKR)	5/15
Acetabular shelf procedure (ASP)	4/15
Arthroscopy knee	4/15
Neer-plasty shoulder	4/15

score of Z = 5.20 at $\theta = 0$ for marker D2S220. Markers D2S2346 and D2S305 on the centromeric side and D2S390 on the telomeric side were the first markers that showed recombinations in the linkage analysis (pair-wise LOD score $-\infty$).

Table IV. Two point LOD scores between BHMED and markers flanking the EDM1, EDM2 and EDM3 loci.

	Recombination fractions (cM)						
Marker	0	0.01	0.05	0.10	0.20	0.30	0.40
D19S226	$-\infty$	-1.61	-0.93	-0.62	-0.30	-0.12	-0.03
D19S414	$-\infty$	-1.01	-0.41	-0.22	-0.07	-0.03	-0.05
D1S255	$-\infty$	-3.12	-1.49	-0.81	-0.28	-0.10	-0.03
D1S2797	$-\infty$	-5.73	-3.01	-1.90	-0.90	-0.41	-0.14
D20S196	$-\infty$	-9.27	-4.59	-2.73	-1.15	-0.47	-0.16
D20S100	$-\infty$	-7.01	-3.58	-2.16	-0.90	-0.33	-0.07
D20S173	$-\infty$	-7.90	-3.91	-2.34	-0.99	-0.39	-0.10

Table V. Pairwise LOD score at various recombination fractions between BHMED and markers on chromosome 2p.

	Recombinant fractions (cM)						
Marker	0	0.01	0.05	0.1	0.2	0.3	0.4
D2S2346	- ∞	0.75	1.81	1.97	1.66	1.05	0.39
D2S305	- ∞	1.53	1.92	1.83	1.32	0.75	0.31
D2S2150	2.19	2.18	2.10	1.95	1.57	1.11	0.58
D2S220	5.20	5.12	4.76	4.30	3.28	2.15	0.93
D2S2221	3.91	3.84	3.55	3.17	2.38	1.53	0.65
D2S73	2.58	2.55	2.39	2.19	1.75	1.25	0.67
D2S390	- ∞	-0.50	0.61	0.84	0.64	0.13	-0.25
D2S146	0.84	0.82	0.76	0.68	0.52	0.37	0.20

Recently, mutations in the matrilin-3 *(MATN-3)* gene were identified in patients with MED (Chapman et al., 2001). This gene maps within the genetic interval determined by the linkage study of this family. After determining the intron-exon

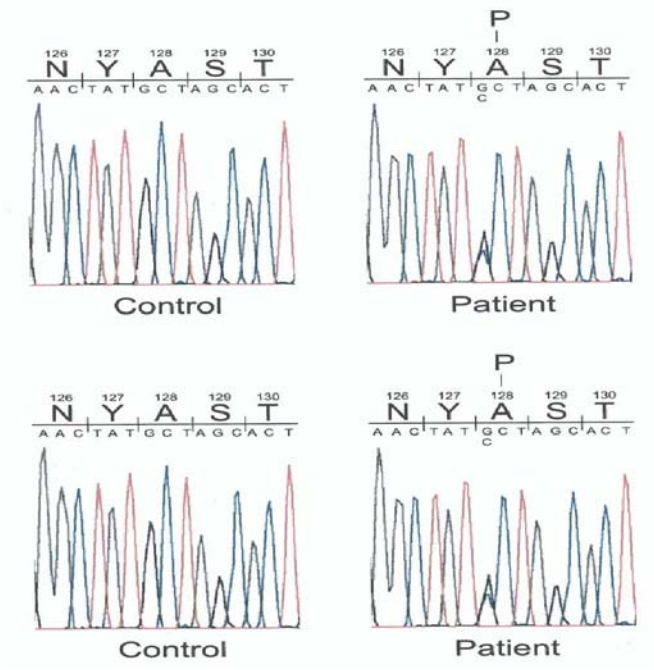

Figure 4. Sequence electropherograms of mutations found in BHMED. Both normal (left) and mutated (right) and amino acid sequences are shown. Right: The hetero-zygous base change G382C in exon 2 resulting in an amino acid substitution of alanine to proline at codon 128 (A128P).

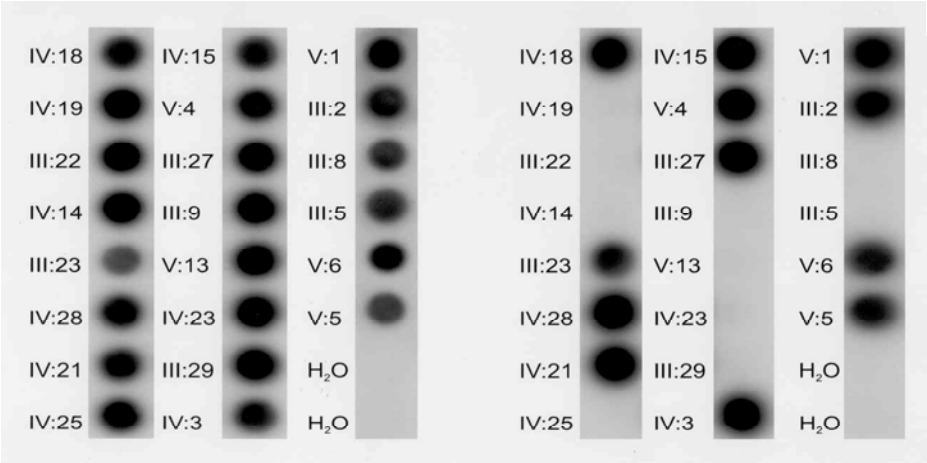

Figure 5. Results of the allele specific oligohybridization (ASO) for the heterozygous base change, G382C in exon 2, from the coding region (reference sequence XM_048680) resulting in an amino acid substitution of an alanine to a proline A128P at codon 128 (A128P). The three colums on the left are the results of the hybridization with the 'wild-type' oligonucleotide. The three colums on the right are the results of the hybridization with the mutant oligonucleotide. Each dot represents a hybridization signal of a DNA sample. Inividual numbers given on the left of each dot and correspondent with the number given in figure 1. Each individual carried at least one normal allele and all affected individuals also carried a mutant allele (the three rows on the left).

boundaries of the *MATN-3* gene, using available sequence databases, the coding region including intron-exon boundaries was sequenced on genomic DNA in a patient and an unaffected individual from the family.

We detected a heterozygous base change, G382C in exon 2, from the coding region (reference sequence XM_048680) resulting in an amino acid substitution of an alanine to a proline A128P at codon 128 (A128P) (Fig. 4). Allele Specific Oligo Hybridization (ASO) confirmed this mutation (Fig. 5). The mutation occurred in a domain which is highly conserved through a number of different species. That the

111	DTLDIGPADTRVAVVNYASTVKIEFQLQAY 140	matrilin-3	Homo sapiens
106	DTLDIGATDTRVAVVNYASTVKIEFQLNTY 135	matrilin-3	Mus musculus
82	DTLDVGERTTRVAVMNYASTVKVEFPLRTY 111	matrilin-3	Gallus gallus
69	ESLDVGPNATRVGMVNYASTVKQEFSLRAH 98	matrilin-1	Homo sapiens
73	ESLDVGPNATRVGLVNYASTVKPEFPLRAH 102	matrilin-1	Mus musculus
69	EGLDVGPNSTRVGVINYASAVKNEFSLKTH 96	matrilin-1	Gallus gallus
87	QFLDIGPDVTRVGLLQYGSTVKNEFSLKTF 114	matrilin-2	Homo sapiens
64	RGLNVGPNATRVGVIQYSSQVQSVFPLRAF 91	matrilin-4	Homo sapiens

Figure 6. Comparison between the human sequence of alanine 128 and that of two other species, house mouse and domestic chicken, shows that alanine 128 is a conserved amino acid along with the mutations in the MATNs identified to date.

human sequence of alanine 128 is indeed a conserved amino acid in humans compared with animals is shown in figure 6, in which the mutations identified to date in the MATNs is pointed out. In addition, the mutation segregated with the disease all available diagnosed relatives, and was not seen in 187 control chromosomes from the general Dutch population.

DISCUSSION

In this study we analyzed the genetic, clinical and radiological features of a skeletal dysplasia previously described by Elsbach in 1959. Elsbach allocated the name bilateral hereditary micro-epiphyseal dysplasia (BHMED) to this condition (Elsbach, 1959). Some authors (Bailey, 1973; McKusick, 1992) argued that this family might be classified as a type of multiple epiphyseal dysplasia (MED). We found the clinical picture in this family and especially the radiological features consistently different from common MED and to be unique in its presentation. To

date, four different genes have been identified in autosomal dominant variants of MED: EDM1 on chromosome 19p13.1, EDM2 on 1p33-p32.2, EDM3 on 20q13.3 and MATN-3 on 2p24-p23 (Chapman et al., 2001; Mortier et al., 2001). An autosomal recessive form of MED (EDM4) is localized on chromosome 5q32-q33.1 and was excluded because of a different mode of inheritance.

Our study confirms the role of MATN-3 in autosomal dominant MED-like skeletal dysplasia as suggested by Chapman (2001) and Mortier (2001). The 4-generation family they described briefly, showed slightly different abnormalities. In the pelvis of some patients, the hip was only unilaterally involved. In our 6-generation BHMED family, all affected joints are bilaterally involved. In EDM3, Paassilta et al. (1999) described a family without complaints of hip and knee, and Bönneman et al. (2000) described a family without complaints of the hip, but predominantly of the knee, whereas there was no limitation of joint function or radiological abnormality of hip and knee.

In these EDM3 families, myopathy and weakness of the lower leg, characterized by the Gower-sign, was reported.

In EDM1 and EDM2, several authors (Oehlmann et al., 1994; Deere et al., 1995; Briggs et al., 1995; Ballo et al., 1997; Ikegawa et al., 1998) described radiological abnormalities of the spine, mostly the lumbar spine.

Recently, a new mutation is described in the collagen IX gene, COL9A1, which codes for the alpha 1 (IX) chain. The results of this study show that mutations in COL9A1 can cause MED, but they also suggest that mutations in COL9A1, COL9A2, COL9A3, COMP and DTDST are not the major causes of MED and that there exists at least one additional locus (Czarny-Ratajczak et al., 2001).

In all these types of EDM, complaints start in early childhood and stabilize during adolescence. In adulthood, complaints in BHMED worsen due to early-onset osteoarthritis. In the Elsbach family, mainly the hip and knee joint were

bilaterally affected and painful, whereas in EDM3 also the elbow and ankle are clinically involved (Bönneman et al., 2000). Affected BHMED-individuals had a proportionally reduced total body height. The affected individuals had a smaller total body height than unaffected first-degree relatives. However, unaffected first-degree relatives in this BHMED family also had lower than average total body height which is most probably due to (epi-)genetic factors like height-related partner selection (Hensley 1994; Roscoe et al., 1987; Mostert et al., 2002b).

Brachydactyly is most characteristic of the autosomal recessive form of MED (EDM4) (Gamboa and Lisker, 1974; Superti-Furga et al., 1999). Short hands with brachydactyly, as often seen in EDM1 and EDM2 (Oehlmann et al., 1994; Briggs et al., 1995; Leeds, 1960; Mourik van et al., 1993) were not reported in BHMED. In the family described here, we found disproportionally shortened MC IV and V (Mostert et al., 2002a).

The clinical features in this family with BHMED represent a consistent and distinct phenotype with differences in comparison with a number of other EDMs.

The finding of a pathogenic mutation in MATN-3, which completely segregated with the clinically affected status, demonstrates that BHMED is indeed a variant of MED. The position of the mutation found in this family (A128P) is within the vWFA domain of MATN-3. The alanine residue at this position is conserved in other species. The other mutations so far described are located within the same domain (V194D and R121D). The function of the vWFA domain is largely unknown but there is evidence that the metal ion-dependent adhesion site motif of the equivalent vWFA domain of matrilin-1 associates with the ECM network (Chen et al., 1999).

In this study, we confirmed that BHMED originally described by Elsbach in 1959 is indeed a distinct clinical entity by means of a pathogenic matrilin-3 mutation in addition to extensive clinical and radiological examination. Further

studies are needed in order to explore the complete phenotypic spectrum of MED and MED-like disorders associated with matrilin-3 mutations.

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REFERENCES

Bailey JA. Disproportionate short stature. Differential diagnosis and management. First Edn. Philadelphia: W.B. Saunders Co., 1973;429.

Ballo R, Briggs MD, Cohn DH, Knowlton RG, Beighton PH, Ramesar RS. Multiple epiphyseal dysplasia, Ribbing type: A novel point mutation in the COMP gene in a South African family. Am J Med Genet 1997;68:369–400.

Bönnemann CG, Cox GF, Shapiro F, Wu J-J, Feener CA, Thompson TG, Anthony DC, Eyre DR, Darras BT, Kunkel LM. A mutation in the alpha 3 chain of type IX collagen causes autosomal dominant multiple epiphyseal dysplasia with mild myopathy. Proc Natl Acad Sci USA 2000;97:1212–1217.

Briggs MD, Hoffman SMG, King LM, Olsen AS, Mohrenweiser H, Leroy JG, Mortier GR, Rimoin DL, Lachman RS, Gaines ES Cekleniak JA, Knowlton RG, Cohn DH. Pseudoachondroplasia and Multiple Epiphyseal Dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nature Genet 1995;10:330–336.

Central institution for statistics (CBS). Vademecum gezondheidsstatistiek. Aspects of health and disease in the population. Edition 1998. Statistics Netherlands, Voorburg/Heerlen, Holland, 302–317.

Chapman KL, Mortier GR, Chapman K, Loughlin J, Grant ME, Briggs MD. Mutations in the region encoding the von Willebrandfactor A domain of matrilin-3 are associated with multiple epiphyseal dysplasia. Nature Genet 2001;28:393–396.

Chen Q, Zhang Y, Johnson DM, Goetinck PF, 1999,: Assembly of a novel cartilage matrix protein filamentous network: molecular basis of differential requirement of von Willebrand factor A domains. Mol Biol Cell 1999;10:2149–2162.

Czarny-Ratajczak M, Lohiniva J, Rogala P, KozlowskiK, Perala M, Carter L, Spector TD, Kolodziej L, Seppanen U, Glazar R, Krolewski J, Latos-Bielenska A, Ala-Kokko L. A mutation in COL9A1 causes multiple epiphyseal dysplasia: further evidence for locus heterogeneity. Am J Hum Genet 2001;69:969–980.

Deere M, Blanton SH, Scott CI, Langer LO, Pauli RM, Hecht JT. Genetic heterogenicity in Multiple Epiphyseal Dysplasia. Am J Hum Genet 1995;56:698–704.

Doege TC, Houston TP. American Medical Association: Guides to the Evaluation of Permanent Impairment. Fourth Edn. Chicago: American Medical Association, 1993.

Elsbach L. Bilateral Hereditary Micro Epiphysial Dysplasia of the hips. J Bone Joint Surg [Br] 1959;41-B:514–523.

Gamboa I, Lisker R. Multiple Epiphyseal Dysplasia tarda: a family with autosomal recessive inheritance. Clin Genet 1974;6:15–19.

Hegenbarth R. Special factors governing x-rays examination of the hip in children. Roentenblatter 1980;33:18–30.

Hensley WE. Height as a basis for interpersonal attraction. Adolescence 1994;29:469-474.

Ikegawa S, Ohashi H, Nishimura G, Kim KC, Sannohe A, Kimizuka M, Fukushima Y, Nagai T, Nakamura Y. Novel and recurrent COMP (cartilage oligomeric matrix protein) mutations in pseudoachondroplasia and Multiple Epiphyseal Dysplasia. Hum Genet 1998;103:633–638.

Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 1984;36:460–465.

Leeds NE. Epiphyseal dysplasia multiplex. Am J Roentgen 1960;84:506–510.

McKusick VA. Mendelian Inheritance in man. A catalog of human genes and genetic disorders. Vol. 2, Eleventh Edn. Baltimore: Johns Hopkins Co., 1992;484-485.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.

Mortier GR, Chapman K, Leroy JL, Briggs MD. Clinical and radiologic features of multiple epiphyseal dysplasia not linked to the COMP or type IX collagen genes. Eur J Hum Genet 2001;9:606–612.

Mostert AK, Dijkstra PF, Horn van JR, Jansen BRH, Heutink P, Lindhout D. Radiological features of bilateral hereditary micro-epiphyseal dysplasia – A distinct entity in the skeletal dysplasias – RöFo Fortschr Geb Röntgenstr Neuen Bildgeb Verfahr 2002a;174:887–892.

Mostert AK, Jansen BRH, Dijkstra PF, Wesby-van Swaay E, Horn van JR, Heutink P, Lindhout D. Bilateral hereditary micro-epiphyseal dysplasia. Further delineation of the phenotype with 40 years follow-up. Int Orthop 2002b;26:188–193.

Mourik van JBA, Schaap C, Nollen AJG. Een Nederlandse familie met erfelijke gewrichtsklachten; Multipele Epiphysaire Dysplasie. Ned Tijdschr Geneeskd 1993;37:32–36.

Oehlmann R, Summerville GP, Yeh G, Weaver EJ, Jimenez SA, Knowlton RG. Genetic linkage mapping of Multiple Epiphyseal Dysplasia to the pericentromeric region of chromosome 19. Am J Hum Genet 1994;54:3–10.

Paassilta P, Lohiniva J, Annunen S, Bonaventure J, Le Merrer M, Pai L, Ala-Kokko L. COL9A3: A third locus for Multiple Epiphyseal Dysplasia. Am J Hum Genet 1999;64:1036–1044.

Roscoe B, Diana MS, Brooks RH. Early, middle and late adolecents' views on dating and factors influencing partner selection. Adolescence 1987;22:59–68.

Stevens PM, Coleman SS. Coxa breva: Its pathogenesis and a rationale for its management. J Pediatr Orthop 1985;5:515–521.

Superti-Furga A, Neumann L, Riebel T, Eich G, Steinmann B, Spranger J, Kunze J. Recessively inherited Multiple Epiphyseal Dysplasia with normal stature, club foot, and double layered patella caused by a DTDST mutation. J Med Genet 1999;36:621–624.

4.4. METACARPOPHALANGEAL PATTERN (MCPP) PROFILE ANALYSIS IN A DUTCH FAMILY WITH BILATERAL HEREDITARY MICRO-EPIPHYSEAL DYSPLASIA LINKED TO MATRILIN-3

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ABSTRACT

Bilateral hereditary micro-epiphyseal dysplasia (BHMED) is a rare skeletal disorder, first described by Elsbach (1959). We studied the extended BHMED family again in order to determine whether this family indeed represents a separate entity to be differentiated from other skeletal dysplasias, especially the multiple epiphyseal dysplasias (MEDs).

The metacarpophalangeal pattern (MCPP) profile analysis is a well-recognized valuable tool to detect changes in the length of hand bones on plain X-ray films in various birth defects, skeletal dysplasias and other hand deformities. In this paper we present the results of our measurements, showing statistically significant differences in Q-scores of BHMED-affected individuals compared with MED-affected individuals and controls. The MCPP profile analysis plots of the measurements showed a curve, characteristic for BHMED, compared with unaffected relatives and patients with EDM2.

We suggest that MCPP profile analysis will be applied more systematically in the clinical and genetic analysis of MED.

In a previous study, we demonstrated that the disorder in this family can be regarded as a clinically distict variant of MED due to a matrilin-3 mutation. In view of subtle hand abnormalities we investigate the value of MCPP profile analysis in clinical diagnosis of affected and unaffected family members using the molecular diagnosis as gold standard, and compared our findings with MCPP profile analyses from a family with EDM2 (n=10).

INTRODUCTION

Bilateral hereditary micro-epiphyseal dysplasia (BHMED) is a rare skeletal dysplasia with an autosomal dominant mode of inheritance with high penetrance, reported by Elsbach in a Dutch family [1].

We re-examined the extended family according to a standard protocol. In view of subtle hand abnormalities seen in BHMED, we quantified the abnormalities by performing MCPP profile analyses.

During the first half of the last century, different methods of radiographic measurements of the hand skeleton were developed [2,3]. In the 1970s, the first statistical data on length of the hand bones for children of all ages and adults became available from White American [4], Hungarian [5], and Venezuelan populations [6]. The normal values for Japanese [7] and Nigerian population [8] followed.

The metacarpophalangeal pattern (MCPP) profile analysis was developed by Poznanski et al. [9]. The MCPP profile analyses is based on measurements of the length of each of the 19 tubular bones of the hand on a postero-anterior (PA) X-ray, and comparing these lengths with the standard of the normal population according to age and sex. The results of this analysis can be visualised in the form of a graph, a so-called 'MCPP-plot' representing relationships between the lengths of metacarpal bones and phalanges compared to a reference population.

Normally, the MCPP profile remains more or less the same for one individual throughout life. In most normal as well as bilaterally affected individuals there is only minimal asymmetry between the left and the right hand, which makes it sufficient to use one hand for the measurements [10]. Familial similarities can be observed in normal families as well as in those with an inherited malformation. MCPP profile analysis has proved to be a valuable method to detect absolute as

well as proportional alterations in the length of the hand bones in various birth defects. For several congenital malformation syndromes the pattern profile appears to be specific [10].

The purpose of this paper is to report on the results of a study evaluating the use of MCPP profile analysis in the delineation and recognition of BHMED-specific hand abnormalities, compared with unaffected family members and patients from a family with EDM2.

Previously we demonstrated that the disorder in the family designated BHMED by Elsbach (1959) is a clinically and radiologically distinct variant of MED due to a matrilin-3 mutation (Mostert et al., 2003). Since we observed subtle abnormalities of the hand, we investigated the value of MCPP profile analysis in the clinical diagnosis of this MED variant while using the matrilin-3 mutation analysis as a golden standard.

MATERIALS AND METHODS

Patients

All affected and unaffected living BHMED family members were examined in a standard way including medical history, physical examination and radiology. Additional laboratory examination was performed in some cases and samples were taken for genetic analysis.

Concerning the hands, we made standardised X-rays of both hands of ten clinically and radiologically BHMED-affected relatives and of three clinically and radiologically unaffected individuals. We included only family members above the age of 16 in order to reduce age-related variation [12]. The focus-film distance was

1.15 m. and the hands were directly placed on the cassette, thus the magnification in all subjects was the same. All physes were closed.

In addition, ten affected MED patients of an EDM2 family were radiologically evaluated in the same way. As controls, we used the measurements of 45 healthy Dutch persons without any known skeletal abnormality. With these radiographs we performed metacarpophalangeal pattern (MCPP) profile plotting. The method consists of measuring the length of the 19 tubular bones of the hand and comparing this length with a standard of the normal population as the reference. The standardised bone lengths in Z-scores will be calculated using the Poznanski data for age and sex stratified bone lengths of White Americans [9]:

$$Z_i = (\text{bone length}_i - \text{reference length}_i) / SD_i \quad (i = \text{the bone number}).$$

The zero-line indicates the mean of the normal population (or reference line). The Z-scores will be plotted against the individual bone number 1 to 19 representing the first metacarpal (MC1) to the distal phalanx (DPh5).

The spread of the tabulated values of the reference standard deviations was a disadvantage. Furthermore, references were only available in relatively large intervals. It is also clear that the radiographs published in medical articles are of a different size than normal life-size radiographs. These were reasons for introducing the Q-score [13,14]. The Q-score is the ^{10}logarithm of the quotient of the length of the hand bone from a patient and the reference length for that bone.

$$Q = {}^{10}\log (\text{bone length}_i / \text{reference length}_i) \quad (i = \text{the bone number}).$$

The reference length is calculated by a formula to adjust the reference to the exact age of the patient. The length of the patient bones in the Q-score can be represented as a percentage of the *Reference length = 100 x (bone length / reference length) – 100*, adjusted to age and gender. Now also published X-rays can be measured, as the Q-plot is a relative measurement tool.

In the Q-plot, the numbers on the X-axis represent also the hand bones listed from the first metacarpal (MC1) to distal phalanx (DPh5). The representation of the Y-axis numbers is in percentages, that is the length of the metacarpals and phalanges is calculated as the difference of the length of these bones from the normal population (the reference length), as calculated by the Q-score.

It is noted that above the age of 20 the Z- and the Q-score do not differ significantly from each other [12]. We present both scores since Q-scores are more appropriate for statistical analysis and Z-scores for comparison with published values.

STATISTICAL ANALYSIS

For an overall comparison of MCPP patterns of the different patients groups, Pearson correlation coefficients were calculated. Significance testing on differences between the patient groups (in order of MCPP bone lengths) was performed using non-parametric one analysis of variance using the Kruskal-Wallis test. In addition, two sample comparisons were made using two sample t-tests or Wilcoxon rank-sum tests (two-sided tests), depending on the distribution of the variables in the comparison, to try to identify where differences between groups were.

A p-value <0.05 was considered significant. However, since in the additional comparisons of the individual MCPP bones no multiple comparison correction was incorporated, the p-values of these tests should be interpreted descriptively.

Figure 1. PA-view of both hands of a 52-year-old affected male.

RESULTS

In BHMED affected family members, X-rays of the wrist showed an 'ulna plus' with slight radial drift of the carpus and flattening of the ulnar part of the radial epiphysis. X-rays of the hands demonstrated indeed short metacarpals, but also a shortening of the proximal and midphalanges. The distal phalanges seemed to have relative normal lengths. There were no deformities (Figure 1).

The Z- and Q-plots were composed of the data, gathered by the measurements of the 19 individual hand bones. The mean length of one bone in one group is figured out in the plots. The unaffected BHMED persons did not have different values compared to the randomly selected Dutch controls in the general population,

matched for age and gender [15]. The Z-plots (Figure 2) and Q-plots (Figure 3) of the affected family members show nearly the same profile. Both plots show a remarkable and consistent profile in the BHMED affected family members. It is not the exact height of the curve, which is most important, but the profile that results from the individual lengthening or shortening of the bones [14].

Hand bone lengths of BHMED patients, MED (EDM2) patients and healthy controls were compared using the Wilcoxon rank-sum tests. The clinical observation of a relatively short metacarpal (MC) of the fourth and fifth digit when clenching the fists in the BHMED patients is also illustrated in the Z- and Q- plots, as MC2 and MC3 are less shorter than MC4 and MC5 compared to the normal population or MED (Figures 2 and 3).

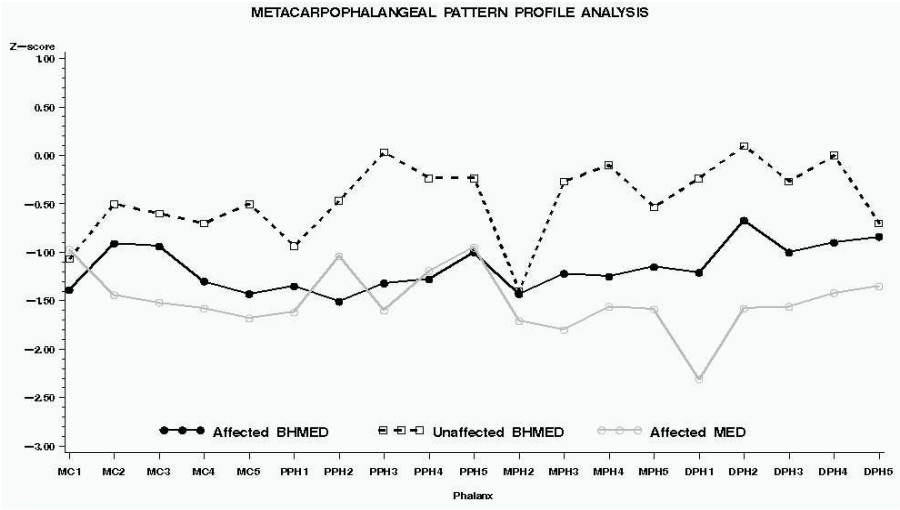

Figure 2 (Z-score). Mean Z-scores of the ten BHMED affected members of the family (●–●–●), the three unaffected family members (□–□–□) and the ten MED (EDM2) affected family members (○--○--○). The numbers on the X-axis represent the hand bones. They are listed as Metacarpal bones 1 to 5 (MC1 to MC5), Proximal phalanges 1 to 5 (PPh1 to PPh5), Middle phalanges 2 to 5 (MPh2 to MPh5) and Distal Phalanges 1 to 5 (DPh1 to DPh5). The numbers on the Y-axis represent the Z-scores for each particular bone. The zero line represents the mean of the healthy population (controls).

In both the affected and the unaffected BHMED family members, the length of MPh2 is shorter than MPh3, MPh4 and MPh5 (Figures 2 and 3).

The outcomes should be interpreted descriptively, because only pre-planned comparisons should be used for conclusions concerning significant differences.

Significant differences were observed between the BHMED-affected family members and the Dutch controls and between the affected-MED (EDM2) persons. All reported p-values were two-sided (Table I).

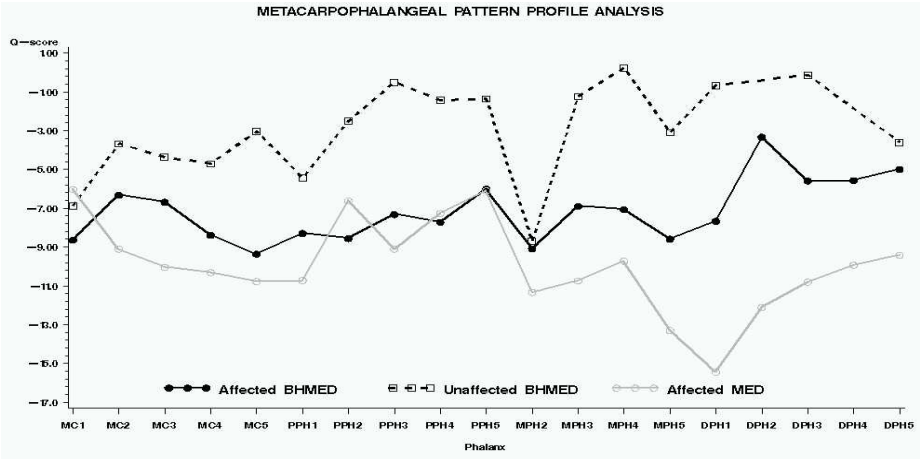

Figure 3 (Q-score). Mean Q-scores of the ten BHMED affected members of the family (●–●–●), the three unaffected family members (□–□–□) and the ten MED (EDM2) affected family members (○--○--○). The numbers on the X-axis represent the hand bones. They are listed as Metacarpal bones 1 to 5 (MC1 to MC5), Proximal phalanges 1 to 5 (PPh1 to PPh5), Middle phalanges 2 to 5 (MPh2 to MPh5) and Distal Phalanges 1 to 5 (DPh1 to DPh5). The numbers on the Y-axis (logarithm-scale) represent the Q-scores in percentages for each particular bone. The zero line represents the mean of the healthy population (controls).

Table I. The results of the measurements of the 19 handbones between the BHMED-affected family members, MED-affected (EDM2) family members and the Dutch controls (Kruskal-Wallis test).

		Affected BHMED	Affected MED	Normal population	Total	p-value
MC_1 value	Mean (±sem)	-8.64 (±1.45)	-6.03 (±1.75)	-1.39 (±0.96)	-3.22 (±0.82)	0.002
	Median	-8.00	-5.75	-2.10	-3.20	
	Q1–Q3	-12.10 – -5.60	-10.90 – -2.80	-5.90 – 2.90	-8.00 – 0.80	
	Min - max	-16.80 – -1.80	-15.30 – 3.40	-12.90 – 14.70	-16.80 –14.70	
	N	10	10	45	65	
MC_2 value	Mean (±sem)	-6.32 (±1.49)	-9.10 (±1.95)	-1.85 (±1.07)	-3.65 (±0.90)	0.006
	Median	-6.50	-9.85	-0.80	-4.00	
	Q1 – Q3	-11.00 – -1.60	-11.80 – -8.30	-7.20 – 2.70	-9.00 – 1.60	
	Min - max	-12.50 – 0.90	-17.10 – 3.80	-18.40 – 13.40	-18.40 –13.40	
	N	10	10	45	65	
MC_3 value	Mean (±sem)	-6.68 (±1.53)	-10.04 (±2.18)	-1.50 (±1.08)	-3.61 (±0.94)	0.001
	Median	-6.85	-10.90	-2.50	-4.10	
	Q1– Q3	-9.50– -5.10	-16.00 – -5.00	-4.90 – 3.60	-7.40 – 1.40	
	Min - max	-15.20 – 2.00	-17.20 – 4.90	-18.00 – 15.60	-18.00 –15.60	
	N	10	10	45	65	
MC_4 value	Mean (±sem)	-8.39 (±1.56)	-10.31 (±2.34)	-1.73 (±1.11)	-4.08 (±0.98)	<0.001
	Median	-9.65	-11.90	-1.90	-3.00	
	Q1– Q3	-11.00 – -3.20	-16.40 – -4.70	-5.40 – 2.60	-10.90 – 1.10	
	Min - max	-16.70 – -2.10	-17.60 – 4.40	-18.40 – 14.00	-18.40 –14.00	
	N					
MC_5 value	Mean (±sem)	-9.36 (±1.37)	-10.77 (±2.31)	-1.91 (±1.20)	-4.42 (±1.03)	<0.001
	Median	-10.20	-10.95	-1.80	-3.60	
	Q1– Q3	-12.60 – -8.20	-14.90 – -7.80	-4.60 – 3.00	-9.20 – 0.20	
	Min - max	-14.10 – 0.20	-22.50 – 5.30	-34.00 – 14.50	-34.00 – 14.50	
	N	10	10	45	65	
PPh_1 value	Mean (±sem)	-8.29 (±2.42)	-10.72 (±3.56)	-2.57 (±1.09)	-4.70 (±1.07)	0.007
	Median	-9.55	-12.70	-1.70	-3.90	
	Q1– Q3	-13.50 – -5.70	-15.30 – -2.90	-8.00 – 1.50	-10.90 – 0.60	
	Min - max	-18.70 – 5.40	-32.00 – 10.30	-15.80 – 17.40	-32.00– 17.40	
	N	10	10	45	65	
PPh_2 value	Mean (±sem)	-8.56 (±1.65)	-6.61 (±2.65)	-1.54 (±1.11)	-3.40 (±0.96)	0.002
	Median	-10.75	-8.40	-1.60	-3.50	
	Q1– Q3	-12.00 – -7.10	-12.30 – -4.40	-5.60 – 1.90	-8.60 – 0.60	
	Min - max	-13.30 – 3.40	-13.50 – 14.50	-17.20 – 18.00	-17.20 – 18.00	
	N	10	10	45	65	
PPh_3 value	Mean (±sem)	-7.32 (±1.31)	-9.10 (±2.28)	-1.27 (±1.10)	-3.41 (±0.95)	<0.001
	Median	-8.60	-10.70	0.30	-4.20	
	Q1– Q3	-10.70 – -4.60	-13.40 – -6.20	-5.30 – 2.10	-8.70 – 1.40	
	Min - max	-11.30 – 1.50	-17.70 – 8.40	-18.00 – 18.30	-18.00 – 18.30	
	N	10	10	45	65	
PPh_4 value	Mean (±sem)	-7.72 (±1.60)	-7.26 (±2.66)	-1.15 (±1.07)	-3.10 (±0.94)	0.004
	Median	-9.95	-10.75	-0.60	-3.00	
	Q1 – Q3	-11.60 – -4.20	-12.10 – -2.00	-5.00 – 3.30	-9.90 – 1.90	
	Min - max	-11.70 – 2.50	-17.30 – 11.60	-17.70 – 16.90	-17.70 – 16.90	
	N	10	10	45	65	
PPh_5 value	Mean (±sem)	-6.01 (±1.83)	-6.09 (±2.47)	-2.28 (±1.04)	-3.44 (±0.88)	0.066
	Median	-8.00	-9.00	-2.00	-3.00	
	Q1 – Q3	-11.10 – -0.70	-10.50 – -3.80	-7.00 – 1.10	-9.70 – 1.10	
	Min - max	-11.70 – 3.90	-13.90 – 12.20	-17.50 – 13.30	-17.50 –13.30	
	N	10	10	45	65	

Continued on p. 124

Table I continued.

		Affected BHMED	Affected MED	Normal population	Total	p-value
MPh_2 value	Mean (±sem)	-9.08 (±1.86)	-11.33 (±1.79)	-4.40 (±1.22)	-6.19 (±0.99)	0.018
	Median	-9.90	-12.35	-3.20	-6.30	
	Q1 –Q3	-13.90 – -2.80	-15.70 – -8.80	-9.70 – 1.40	-12.30 – -1.10	
	Min - max	-17.40 – 0.10	-17.80 – 2.10	-21.50 – 13.30	-21.50 –13.30	
	N	10	10	45	65	
MPh_3 value	Mean (±sem)	-6.90 (±1.70)	-10.74 (±2.08)	-2.68 (±1.24)	-4.57 (±1.02)	0.010
	Median	-7.35	-10.95	-1.50	-4.80	
	Q1 – Q3	-9.90 – -3.70	-15.30 – -8.10	-8.60 – 3.00	-10.40 – 1.30	
	Min - max	-14.40 – 3.10	-19.00 – 3.30	-20.10 – 12.70	-20.10 –12.70	
	N	10	10	45	65	
MPh_4 value	Mean (±sem)	-7.07 (±2.00)	-9.72 (±2.28)	-2.18 (±1.12)	-4.09 (±0.97)	0.007
	Median	-7.80	-10.70	-1.50	-5.30	
	Q1– Q3	-11.00 – -5.30	-15.00 – -6.00	-7.30 – 2.90	-9.10 – 2.30	
	Min - max	-15.70 – 4.90	-19.70 – 5.00	-20.90 – 13.20	-20.90– 13.20	
	N	10	10	45	65	
MPh_5 value	Mean (±sem)	-8.58 (±2.26)	-13.29 (±4.22)	-3.83 (±1.30)	-6.01 (±1.22)	0.013
	Median	-8.75	-15.20	-3.20	-4.90	
	Q1– Q3	-14.10 – -2.80	-17.40 – -3.20	-8.60 – 3.20	-14.00 – 2.10	
	Min - max	-18.40 – 2.00	-41.40 – 7.60	-24.70 – 12.90	-41.40 – 12.90	
	N	10	10	45	65	
DPh_1 value	Mean (±sem)	-7.67 (±2.05)	-15.44 (±2.27)	-5.00 (±1.57)	-7.02 (±1.26)	0.002
	Median	-7.85	-16.30	-4.30	-6.90	
	Q1 - Q3	-13.60 – -2.70	-20.70 – 12.70	-9.00 – 2.50	-12.70 – 0.20	
	Min - max	-16.40 – 3.30	-26.50 – -1.20	-40.50 – 12.20	-40.50 – 12.20	
	N	10	10	45	65	
DPh_2 value	Mean (±sem)	-3.33 (±2.20)	-12.11 (±2.48)	0.36 (±1.25)	-2.12 (±1.13)	<0.001
	Median	-1.70	-12.05	-1.00	-1.30	
	Q1– Q3	-5.90 – 1.30	-17.00 – -5.60	-2.10 – 4.80	-7.50 – 3.00	
	Min - max	-15.30 – 6.80	-27.20 – -0.70	-20.50 – 17.20	-27.20 – 17.20	
	N	10	10	45	65	
DPh_3 value	Mean (±sem)	-5.59 (±1.35)	-10.80 (±2.28)	-1.07 (±1.22)	-3.26 (±1.03)	0.001
	Median	-5.10	-10.95	-2.10	-3.70	
	Q1 – Q3	-7.10 – -3.70	-15.20 – -4.10	-5.90 – 2.70	-8.70 – 2.00	
	Min - max	-12.20 – 2.00	-21.30 – 1.20	-19.20 – 17.30	-21.30 – 17.30	
	N	10	10	45	65	
DPh_4 value	Mean (±sem)	-5.57 (±2.10)	-9.93 (±2.60)	-2.06 (±1.18)	-3.81 (±1.02)	0.015
	Median	-3.70	-9.85	-2.40	-4.00	
	Q1 – Q3	-10.40 – -1.00	-16.90 – -7.10	-6.10 – 3.10	-8.60 – 1.30	
	Min - max	-20.30 – 2.60	-19.80 – 7.30	-18.80 – 13.60	-20.30 – 13.60	
	N	10	10	45	65	
DPh_5 value	Mean (±sem)	-5.00 (±2.94)	-9.41 (±3.44)	-1.16 (±1.20)	-3.02 (±1.13)	0.024
	Median	-0.65	-10.85	-1.40	-1.80	
	Q1 – Q3	-10.20 – 0.90	-14.60 – -1.30	-6.30 – 4.10	-8.20 – 3.10	
	Min - max	-24.60 – 5.70	-29.40 – 9.90	-18.80 – 12.50	-29.40 – 12.50	
	N	10	10	45	65	

DISCUSSION

In BHMED, bilateral simultaneous onset of pain in hip and knee joints, short stature, waddling gait and restricted range of motion of the hip were seen in the affected family members. The hands appeared to be relatively broad and short. However, when clenching the fists, a shortening of the metacarpals (MC), especially MC IV and V was noticed. In our measurements, the Z- and Q-plots confirmed this observation. The plots show a characteristic and consistent profile. The disproportionally shortened MPh2 in affected as well as unaffected BHMED family members strongly suggests that this is a family trait that is independent of the BHMED trait. We also have to take into account that unaffected BHMED family members have a smaller height compared to the normal (Dutch) population, which can be explained by the fact that BHMED patients seem to marry preferentially with persons of smaller height, who make a genetic contribution to the next generation independent of the segregation of the gene for BHMED [16].

MCPP pattern plotting seems limited to the analysis of discriminating affected and unaffected persons within one family. It is not the exact height of the curve, which is most important, but the profile that results from the individual lengthening or shortening of the bones. Characteristic profiles for BHMED are represented in figures 2 and 3.

Discrimination between different dysplasias with the MCPP profiles is possible, but only when the profiles are quite different. Especially in the multiple epiphyseal dysplasia (MED), where short, stubby fingers are seen, the radiological features are different from BHMED. MED shows a more generalised shortening of all bones of the hand, whereas in BHMED the bones of the hand other than MC IV and V are less shortened than in MED, which is in agreement with the clinical observation of disproportionally shortened MC IV and V.

The finding of a pathogenic mutation in MATN-3 in this family (A128P), which completely segregated with the clinical affection status [11], demonstrated that BHMED belongs a variant of MED (i.e. EDM5).

We conclude that MCPP profile analysis comfirmed subtle hand abnormalities in BHMED and suggest that MCPP profile analysis will be more systematically applied in the clinical and genetic analysis of MED and its genetic variants.

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REFERENCES

1. Elsbach L. Bilateral Hereditary Micro-Epiphyseal Dysplasia of the hips. J Bone and Joint Surg [Br] 1959;41B:514–523.
2. Archard MC. Arachnodactylie. Bulletin de la Société médicale des Hopitaux de Paris 1902 ;19:834–840.
3. Parish JG. Radiographic measurements of the skeletal structure of the normal hand. Br J Radiol 1966;39:52–62.
4. Garn SM, Hertzog KP, Poznanski AK, Nagy JM. Metacarpophalangeal length in the evaluation of skeletal malformation. Radiology 1972;105:375–381.
5. Gefferth K. Metrische auswertung der kurzen röhrenknochen der Hand von der Geburt bis zum ende der Pubertät: Längenmasse. Acta Paed Acad Scient Hung 1972:1-3:117–124.
6. Arias CS, Larralde AR. Longitud de metacarpianos y falanges para la poblacion metropolitana de Caracas, de ambos sexos adulta y de 2 a 18 años. Acta Cientificas Venezolana 1980;31:475–484.
7. Matsura S, Tadashi K. Radiographic measurements of metacarpophalangeal lengths in Japanese children. Jap J Hum Genet 1989;34:159–168.
8. Odita JC, Okolo AA, Ukoli F. Normal values for metacarpal and phalangeal lengths in Nigerian children. Skelet Radiol 1991;20:441–445.
9. Poznanski AK, Garn SM, Nagy JM, Gall JC. Metacarpophalangeal pattern profiles in the evaluation of skeletal malformations. Radiology 1972;104:1–11.
10. Poznanski AK. The hand in radiologic diagnosis. Saunders Monographs in clinical radiology, Philadelphia, W.B. Saunders Co, Vol. 4, 2nd edition, 1984.
11. Mostert AK, Dijkstra PF, Jansen BRH, Horn van JR, Graaf de B, Heutink P, Lindhout D. Familial multiple epiphyseal dysplasia due to a matrilin-3 mutation: Further delineation of the phenotype including 40 years follow-up. Am J Med Genet 2003;in press.
12. Dijkstra PF, Venema HW. Metacarpophalangeal pattern profiles: Q-score for ages from 3 years to adult with epiphyses: an update [letter]. Am J Med Genet 1992;43:1041–1043.

13. Dijkstra PF, Venema HW. Metacarpophalangeal pattern profiles: Q-scores for ages from birth to 7 years. Am J Med Genet 1991;40:107–114.
14. Dijkstra PF. Metacarpophalangeal pattern profile analysis. Amsterdam, The Netherlands: University of Amsterdam, Thesis 1993;121–130.
15. Central institution for statistics (CBS). Vademecum gezondheidsstatistiek. Aspects of health and disease in the population. Edition 1998. Voorburg, Holland, 1998;302–317.
16. Mostert AK, Jansen BRH, Dijkstra PF, Wesby-van Swaay E, van Horn JR, Heutink P, Lindhout D. Bilateral hereditary micro-epiphyseal dysplasia: Further delineation of the phenotype with 40 years follow-up. Int Orthop 2002;26(3):188–193.

CHAPTER 5

DISCUSSION

In this study, we attempted to address the following questions:

1. Is BHMED indeed a separate clinical entity among the MEDs?
2. What are the diagnostic radiographic features of BHMED?
3. What is the molecular genetic basis of BHMED?
4. What is the diagnostic value of metacarpophalangeal pattern (MCPP) profile analysis?

Is BHMED indeed a separate clinical entity among the MEDs?

The phenotypical description of Elsbach findings on a Dutch family (1959), 40 years ago, suggested a familial trait, in which an autosomal dominant dys-plasia was the inheritance pattern. He stated that his findings were different from the known MED, especially the radiological findings of small epiphyses of the hip.

We re-examined the extended family and all subjects over 4 years old were included (no subjects below this age were available). Our observations confirm that the disorder in this family belongs to the MED group, but can be distinguished from other MEDs in this group. Special features are early-onset bilateral onset of complaints (pain in hip and knees), and small epiphyses, i.e. ‘micro-epiphyses’. MCPP profile analysis of affected family members further underlined the distinct pattern of skeletal features in this disorder by showing a specific pattern different from the most common form of MED, EDM1.

Thorough clinical and radiological examination of the affected and unaffected family members was needed in order to obtain a reliable clinical phenotype definition and to sort out branches of the family that did not have the disease. In all affected cases, the radiological findings confirmed the clinical findings. It was

important to differentiate specific dysplasia complicated by osteoarthritis from aspecific osteoarthritis without a predisposing dysplasia. The application of a standardized protocol for medical history, physical examination and radiological evaluation was essential in obtaining these results.

A small height may lead towards selection of a partner who also has a smaller height. Therefore we examined also the spouses of affected family members who had offspring, and paid equally well attention to the possibility that a smaller height among spouses may be associated with an increased risk of another skeletal dysplasia gene being married in into the family. We did not find evidence for a second skeletal dysplasia but observed indeed that on average spouses of affected family members were shorter than controls. This may explain why unaffected children of patients in this family also had a shorter height. It suggests that the short stature in BHMED patients as compared to the general population is due to the combined effect of the dominant gene mutation and the polygenetic background of both parents and a possible role of non-random partner selection in quantitative traits like height.

Indeed, the literature confirms the role of height-related partner selection (Hensley, 1994; Roscoe et al., 1987). It is evident that total body height (TBH) is not a distinctive inclusion criterion when defining the affection status in a skeletal dysplasia with mild to moderate effect on final adult height and with no or only limited reduction of reproductive fitness.

What are the diagnostic radiographic features of BHMED?

Although clinical symptoms strongly suggested that the 'Elsbach family' has a distinct form of MED, radiological findings provided more conclusive evidence for BHMED as a separate entity. In BHMED, the well-developed ossification centre of

the epiphyses is small and homogeneous, whereas in MED accessory ossification centres are seen, scattered in the periphery of the cartilaginous epiphyses. With regard to the weight-bearing joints, especially the hip joints, the flattening of the femoral head is due to mechanical stress, probably resulting in secondary degenerative osteoarthritis in later life (see Appendix 1). The ulcera and osteochondritis dissecans-like defects may possibly have the same origin. In BHMED, the metacarpals and phalanges are short, but the distal phalanges not stubby as in MED (Mourik van, 1998). The 'V' wrist joint deformity in MED which is due to hypoplasia of the adjacent portions of the radial and ulnar epiphyses, was not observed in BHMED, although affected subjects showed deformity of the ulnar part of the distal radial epiphysis. In BHMED, the carpal bones are of normal size without irregularities, whereas they are small and irregular in MED.

In the 'Elsbach family', the spine is not affected. In MED, the spine shows minimal flattening and reduction of the vertebral bodies. In the 'Elsbach family' the femoral head was medially flattened in every affected relative, whereas in MED a large variation of abnormalities may be observed ranging from normal to gross deformation.

We used our own developed X/Y ratio scoring system because of the marked reduced height of the intercondylar notch in BHMED-affected patients. The measurements stated by Schlesinger (1986) compare only the width of the distal femoral metaphysis and that of the accompanying epiphysis. The notch height is not measured.

In summary, BHMED shows a unique pattern of radiographic abnormalities that are distinct from common MED. Radiographic examination was an important tool in delineating the phenotype in this family, and may be of great value in differential phenotyping of new MED families that are not yet genetically or molecularly characterized.

What is the molecular genetic basis of BHMED?

After clinical and radiographical phenotyping, linkage analysis in the 'Elsbach family' showed no linkage to previously known loci for autosomal dominant MED, EDM1-3, and revealed linkage to a region harbouring the matrilin-3 gene. Subsequently, a missense mutation was found within the von Willebrand domain of this gene, which was not observed among healthy population controls. Shortly before our discovery, this gene was found to be mutated in the two families reported by Chapman et al. (2001). Although the clinical description of these two reported families is rather scarce, the available information suggests that the phenotypes resemble that of our family and are distinct from common MED. In view of the role of matrilin-3 in the build-up of the extra-cellular matrix as reviewed before, and of what is currently known about the temporospatial expression patterns of matrilin-3, defects of matrilin-3 can indeed be held responsible for the clinical phenotype of BHMED. Detection of other mutations in the different domains of matrilin-3 and analysis of the associated clinical pictures will help to obtain more insight into the role of matrilin-3 in connective tissue assembly and its interplay with other molecules.

What is the diagnostic value of metacarpophalangeal pattern (MCPP) profile analysis?

Many congenital or hereditary disorders are characterized by a subtle pattern of skeletal abnormalities of the hand. This may reflect into small but specific changes of the absolute and relative length of hand bones. The MCPP profile analysis is a useful instrument in making these abnormalities visible as an abnormal pattern and available for interpretation. MCPP profile analysis can be used twofold: to increase the (differential) diagnostic precision and to analyze the segregation pattern within the family. Sometimes it may also be helpful in sorting out features that are related

to the genetic condition or that are representing variants running in the family as a kind of skeletal polymorphism. All of these aspects played a role in this study. We observed a MCPP plot that showed a clear difference from that of EDM2. MCPP profile analysis of available family members gave results that were consistent with the segregation of BHMED according to preset clinical and radiological criteria. This was confirmed by the results of linkage and mutation analysis. In addition, MCPP profile analysis demonstrated that a disproportionately short second digit in this family was due to a short MPh2 not related to BHMED but a feature that independently run through the family and represented a constituent of the genetic family background.

PROSPECTS FOR FUTURE RESEARCH

The identification of gene defects causing a skeletal dysplasia may help patients and families to understand the nature of the disease, comprehend the genetic risks to the offspring, and obtain adequate genetic counselling. This study illustrates the importance of clinical and molecular genetic analysis to the family. One whole branch of the family that was thought to be affected was found to be clinically unaffected. The occurrence of slightly short stature in combination with aspecific osteoarthropathy and heteroanamnestic ‘hear say’ information may have mislead Elsbach in his original study. In addition, one young family member that was thought to be affected clinically was found not to be affected on the basis of radiographic examination and absence of the matrilin-3 mutation confirmed she had not inherited the disease.

As mentioned in the introduction, MED is regarded as a rather rare disorder, even when taken as a group. Its prevalence is estimated as 11.2 per 1 million based on index patients, and 16.3 per million, if affected relatives are included (Wynne-Davies and Gormley, 1985). Later estemates showed a prevalence of 9:100,000 (Taybi and Lachman, 1996). Based on these highly different estimates the total number of persons with MED in the Netherlands may vary between 168–244 and 3600. Thus, every Dutch hospital cares for at least 1–2 to 15 patients with MED. However, hidden behind the non-specific label of 'bilateral premature osteoarthritis', MED may be more common than appreciated. There are a number of 'normal' individuals who, in addition to premature degenerative changes of weight-bearing joints, show involvement of two or more joints and have a history of similar disease in near relatives. This suggests that there are many more patients with milder or less typical forms of disease that share a common molecular mechanism. The extension of our knowledge may go into two directions. First, the currently known loci for autosomal dominant MED represent probably the tip of an iceberg. Second, we have to take into account the possibility that the currently known genes may show slight variations of the coding sequences or the promoter elements that may give rise to modulating effects in more common osteoarthropathies with complex aetiology. This requires larger scale genetic epidemiological studies. Conversely, 'larger' mutations of these genes may induce more severe abnormalities giving rise to clinical phenotypes that are lethal or not consistent with reproduction, and therefore may be associated with sporadic occurrence rather than familial presentation with an autosomal dominant mode of transmission. Clinical analysis of patients with chromosomal microdeletions or partial triploidies of the regions encompassing the MED associated genes may be helpful to explore the effect of haploinsufficiency (through microdeletion) or triple gene dose effect (through partial trisomy). In sporadic patients, precise clinical

phenotyping in combination with large scale testing of functional candidate genes may be needed in order to estimate the role of new mutations in severe cases.

Knowledge of the genes involved in monogenic MED and multifactorial osteoarthropathies will foster our understanding of the molecular basis of these disorders, its environmental risk factors, its prognosis, and ultimately the individualized risk-benefit assessment of different treatments. Comprehension of the molecular mechanism may also lead towards innovative molecular treatment modalities, for example through the design of replacement materials that provide a surface for specific interaction with surrounding human tissues and better integration in the human body.

REFERENCES

Central institution for statistics (CBS). Vademecum gezondheidsstatistiek. Aspects of health and disease in the population. Edition 1998. Voorburg: Holland, 1998;302–317.

Chapman KL, Mortier GR, Chapman K, Loughlin J, Grant ME, Briggs MD. Mutations in the region encoding the von Willebrand factor A domain of matrilin-3 are associated with multiple epiphyseal dysplasia. Nat Genet 2001;28(4):393–396.

Elsbach L. Bilateral hereditary miro-epiphyseal dysplasisa of the hips. J Bone Joint Surg 1959;41B:514–523.

Hensley WE. Height as a basis for interpersonal attraction. Adolescence 1994; 29: 469-474.

Mourik van JBA. Multiple Epiphyseal Dysplasia. A clinical and molecular genetic study. Thesis 1998, Rotterdam.

Roscoe B, Diana MS, Brooks RH. Early, middle and late adolecents' views on dating and factors influencing partner selection. Adolescence 1987;22:59–68.

Schlesinger AE, Poznansli AK, Pudlowski RM, Millar EA. Distal femoral epiphysis: Normal standards for thickness and application to bone dysplasias. Radiology 1986;159:515–519.

Taybi H, Lachman RS. Radiology of Syndroms Metabolic Disorders, and Skeletal Dysplasies, 4th Edn. Mosby, New York , 1996.

Wynne-Davies R, Gormley J. The prevalence of skeletal dysplasias. An estimate of their minimum frequency and the number of patients requiring orthopaedic care. J Bone Joint Surg 1985;67B:133–137.

CHAPTER 6

SUMMARY

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SAMENVATTING

This thesis is based upon a study of a Dutch family with a unique skeletal dysplasia first described by Elsbach in 1959. Clinical features were early onset of bilateral symmetric synchronic complaints of pain in hip and knee, and radiographically small epiphyses of the hip joint. Although Elsbach regarded this disorder as different from common multiple epiphyseal dysplasia, McKusick suggested that it was not different from what was defined later as Fairbanks and Ribbing types of MED. Since the latter disorders were proven to be caused by mutations in the COMP gene, this assumption became amenable to verification or falsification. Furthermore, the family requested genetic counselling and wanted be informed about the genetic risks to the offspring and to obtain more information about the nature of the disease.

The aim of the study (Chapter 2) was to obtain answers to four main questions:

1. Is BHMED indeed a separate clinical entity among the MEDs?
2. What are the diagnostic radiographic features of BHMED?
3. What is the molecular genetic basis of BHMED?
4. What is the diagnostic value of metacarpophalangeal pattern (MCPP) profile analysis?

A review of the literature on MED and MED-relate disorders is given with respect to clinical symptoms (Chapter 3.1), genetics (Chapter 3.2), pathogenesis (Chapter 3.3) and course and treatment (Chapter 3.4).

In Chapter 4.1, the results of the clinical study of the ‘Elsbach family’ are given. The study included the original family including 40 years follow-up period, as well as new family members born since the first report by Elsbach. By application of a standard protocol for clinical and radiological evaluation and personal examination

of all family members, the phenotype in the family was defined more precisely. The findings confirmed Elsbach's conclusion that the disease in this family, designated by him as BHMED, indeed represents a separate entity that can be differentiated from common MED. However, the study also revealed that in one branch of the family the diagnosis of BHMED by Elsbach was incorrect.

Short stature was not only observed in affected patients, but also though to a lesser degree in their healthy siblings and the unaffected parents. This observation made it clear that short stature by it self is not a good clinical criterion for the diagnosis of autosomal dominant skeletal dysplasias with mild shortening of stature and preserved reproductive fitness. Through height-related partner choice, affected as well as unaffected offspring will on average share the same genetic background which is enriched for genetic factors for being short. This also indicated that the effect of the mutation for BHMED on height is probably less than observed by comparing the height of patients with population controls. One has to be aware that this influx of genes for being short probably may have been going on for a number of successive generations.

The radiographic findings were described and analysed in more detail in Chapter 4.2. BHMED could again be distinguished from common MED by a specific combination of features mainly consisting of hip abnormalities characterized by a valgus angle of the femoral neck which is short and thickened, flattening of the head of the femur and the femoral epiphysis especially at the medial part -at the site of the fovea centralis-, and dysplasia of the acetabulum as expressed by a lack of development of the normal teardrop configuration of the medial acetabular wall. The abnormalities of the knee were characterised by dysplastic deformities like flattened and small condyles, especially on the lateral

view in contrast with unaffected family members. In a few cases there was a focus of osteochondritis dissecans and in others an ulcus was seen at the lateral femoral condyle. All affected family members showed a shallow intercondylar notch on the AP view of the knee compared to the unaffected family members. Besides, a number of other or less specific skeletal abnormalities were found. In one family member the clinical diagnosis of BHMED was not confirmed radiographically.

Through linkage analysis three candidate loci, EDM1-3 cold be excluded (Chapter 4.3). A whole genome scan was started which revealed linkage to a region on chromosome 2 harbouring a functional candidate gene, matrilin-3. At this phase, a report by Chapman et al. appeared suggesting that mutations of matrilin-3 indeed were associated with variant forms of MED. The clinical phenotype of these families is not well described but shows certainly a number of similarities with those of the Elsbach family. Mutation analysis of matrilin-3 revealed a previously unreported missense mutation in the von Willebrand domain of this gene (G382C base pair substitution leading to an A128P amino acid substitution). This change concerns an evolutionary highly conserved amino acid. In addition, the mutation was not found in 187 healthy controls. Taken together, it was concluded that this mutation is indeed the genetic cause of BHMED. These findings confirm Elsbach's and our observation that the phenotype in this family is indeed distinct from common MED or Fairbanks and Ribbing type of MED (EDM1), contrary to McKusick's assumption (OMIM, 2002).

The mutation segregated completely with the preset affection status which required fulfilment of the criteria for both clinical and radiographic diagnosis. The mutation was absent in the branch of the family that we diagnosed as unaffected contrary to Elsbach's original description. The mutation was also absent in the patient who showed clinical signs of BHMED but which could not be confirmed

radiographically. This shows the usefulness of supplementary radiological examination for the clinical diagnosis in individual patients and for phenotyping in preparation for molecular genetic analysis.

The MCPP profile observed in BHMED was found to be different from that of EDM2 and was consistent with the clinical diagnosis, the radiological observations and the segregation of the matrilin-3 mutation (Chapter 4.4). By MCPP profile analysis we were also able to determine that a disproportionally shortened MPh2 in affected as well as unaffected BHMED family members was a family trait that is independent of the BHMED trait. This study demonstrated the great power of MCPP profile analysis for phenotypic classification in studies of large family with multiple affected persons. Its differential diagnostic value in individual patients, seen independent from a family or family history, was not a specific subject of this study.

Chapter 5 provides a comprehensive discussion of the results of this thesis and a number of reflections on prospects for the future. The four aims of the study, further clinical delineation of the BHMED phenotype, radiographic characterisation, identification of its molecular genetic basis, and establishing the complimentary value of MCPP profile analysis using the genotype as golden standard were all accomplished.

The study also revealed an interesting role for height-related partner choice on the polygenetic background against which the matrilin-3 gene affects height. Our observations may stimulate the development of models to measure the independent contributions and interactions of major and minor gene effects in quantitative traits in general.

The identification of genes involved in skeletal dysplasia and predisposing to premature osteoarthropathies may also help to gain more insight into the complex developmental and molecular basis of these disorders, to develop new methods of treatment, to select the best treatment in case similar clinical conditions due to different underlying defects respond differently, to identify environmental risk factors that may provide a grip for preventive action, and to provide a sound basis for genetic counselling.

Deze dissertatie is gebaseerd op een onderzoek naar een Nederlandse familie met een unieke skelet dysplasie, die voor het eerst werd beschreven door Elsbach in 1959. De klinische verschijnselen die hij beschreef traden op in de vroege kinderjaren en bestonden uit dubbelzijdig en gelijktijdig optredende, symmetrische klachten van pijn in met name heup en knie met daarbij radiologisch kleine epiphysen van het heupgewricht. Hoewel Elsbach deze bevindingen beschouwde als anders dan die van de bekende multipele epiphyseaire dysplasie (MED), suggereerde McKusick reeds dat deze bevindingen waarschijnlijk niet anders waren dan die later werden gedefinieerd als het Fairbanks en Ribbing type MED. Zodra de laatst genoemde aandoening bleek te worden veroorzaakt door mutaties in het cartilage oligo matrix protein (COMP)-gen, werd deze veronderstelling hanteerbaar voor verificatie of falsificatie. Hierbij kwam dat de familie zelf vragen had over de erfelijkheid en de aard van de aandoening en geïnformeerd wilde worden over de genetische risiko's voor het nageslacht. Bij velen van hen leefde het verhaal dat een verre voorvader in Nederlands-Indië door een krokodil was gebeten, en dat daardoor de aandoening in de familie gekomen was.

Het doel van deze studie (Hoofdstuk 2) was antwoorden te verkrijgen op de volgende vragen:

1. Is bilaterale hereditaire micro-epiphysaire dysplasie (BHMED) inderdaad een aparte klinische entiteit binnen de grotere groep van MEDs?
2. Wat zijn de diagnostische, radiologische kenmerken van BHMED?
3. Wat is de moleculair genetische basis van BHMED?
4. Wat is de diagnostische waarde van metacarpophalangeal pattern (MCPP) profile analysis?

Een literatuurstudie naar MED en MED-gerelateerde aandoeningen wordt gegeven ten aanzien van de klinische symptomen (Hoofdstuk 3.1), de genetica (Hoofdstuk 3.2), de pathogenese (Hoofdstuk 3.3) en beloop en behandeling (Hoofdstuk 3.4).

In Hoofdstuk 4.1 worden de resultaten beschreven van de klinische studie van de 'Elsbach familie'. Deze studie bevat de oorspronkelijke familie, inclusief 40 jaar follow-up, als ook familieleden die na die eerste publicatie van Elsbach zijn geboren. Door gebruik te maken van een gestandaardiseerd protocol voor klinische en radiologische evaluatie en individueel onderzoek van alle familieleden kon het phenotype van de familie nauwkeuriger worden gedefinieerd. De bevindingen bevestigden Elsbach's conclusie dat de aandoening in deze familie, door hem benoemd als BHMED, inderdaad een aparte entiteit vormt die kon worden gedifferentieerd van de bekende MED. Echter, uit ons onderzoek bleek dat hij ook een tak van de familie abusievelijk met BHMED had aangemerkt.

Een korte lichaamslengte werd niet alleen gezien bij aangedane familieleden, maar in mindere mate ook bij niet-aangedane familieleden en de gezonde partners. Deze observatie maakt duidelijk, dat een korte lichaamslengte op zichzelf genomen geen goed klinisch criterium is voor de diagnose van een autosomaal dominante skelet dysplasie met een mild verkorte lichaamslengte en behouden reproductieve 'fitness'. Door 'height-related partner choice' zullen zowel aangedane als ook niet-aangedane familieleden gemiddeld dezelfde genetische achtergrond delen die is verrijkt met genetische factoren voor de kortere lichaamslengte. Dit geeft tevens aan, dat het effect van de mutatie voor BHMED voor wat de lichaamslengte betreft waarschijnlijk minder is dan dat gezien wordt in vergelijkbare gezonde controles en de gegevens van het CBS. Men dient er rekening mee te houden, dat deze influx

van genen voor de kortere lichaamslengte over generaties heen een rol kan hebben gespeeld.

De radiologische bevindingen worden beschreven en specifieker geanalyseerd in Hoofdstuk 4.2. BHMED kan wederom worden onderscheiden van de bekende MED door een specifieke combinatie van kenmerken, die voornamelijk bestaan uit heupafwijkingen, zoals coxa valga met een verkort en verdikt collum femoris, een afplatting van de femurkop, met name aan de mediale zijde van de epiphyse -aan de zijde van de fovea centralis-, en een dysplastisch acetabulum met een matige ontwikkeling van de 'teardrop configuration' aan de mediale acetabulaire wand. De afwijkingen van de knie worden gekarakteriseerd door dysplastische deformiteiten zoals afgeplatte en kleine femurcondylen, met name op de laterale opname, in vergelijking met de niet-aangedane familieleden. In een aantal gevallen werd er een haard van een osteochondritis dissecans gevonden en bij weer anderen werd een ulcus gezien in de laterale femurcondyl. Alle aangedane familieleden vertoonden een vlakke intercondylaire notch op de voor-achterwaartse opname van de knie, vergeleken met de niet aangedane familieleden. Daarnaast werd een aantal minder specifieke skelet afwijkingen gevonden. In één familielid kon de klinische diagnose BHMED radiologisch niet worden bevestigd.

Door middel van linkage analyse konden drie kandidaat loci, EDM1-3 worden uitgesloten (Hoofdstuk 4.3). Vervolgens werd een volledige genoom scan uitgevoerd, die linkage toonde met een regio op chromosoom 2 die op zijn beurt een functioneel kandidaatgen, matrilin-3 (MATN-3), bleek te bevatten. Juist op dat moment verscheen een publicatie door Chapman et al. (2001), die suggereerde dat mutaties van MATN-3 inderdaad geassocieerd waren met varianten van MED. Het klinische phenotype van de door hen onderzochte families was relatief summier

omschreven, maar vertoonde zeker een aantal overeenkomsten met die van de 'Elsbach familie'. Mutatie analyse van matrilin-3 in onze familie toonde een nieuwe missense mutatie aan in het von Willebrand domein van dit gen (G382C basepaar substitutie, resulterend in een A128P aminozuur substitutie). Het betreft een aminozuur dat evolutionair hoog geconserveerd is. Voorts werd deze mutatie niet gevonden in 187 gezonde controle personen. Hieruit werd geconcludeerd dat deze mutatie inderdaad de genetische oorzaak van BHMED is. Deze bevindingen bevestigen Elsbach's en onze waarneming dat het phenotype van de skeletdysplasie in deze familie inderdaad anders is dan dat van de bekendere en vaker voorkomende MED (Fairbanks en Ribbing type) (EDM1). Hiermee is tevens McKusick's (OMIM) aanname dat het om eendere aandoeningen zou gaan weerlegd.

De mutatie segregeerde volledig met de aandoening zoals voorafgaand op grond van klinsche en radiologische kenmerken vastgesteld. De mutatie was niet aanwezig in een tak van de familie waarin wij in tegenstelling met Elsbach's originele beschrijving de diagnose niet konden stellen. De mutatie werd ook niet gezien in de patiënt, die weliswaar klinisch verschijnselen van BHMED vertoonde, maar waarbij dat radiologisch niet kon worden bevestigd. Deze bevinding onderschrijft nog eens de waarde van aanvullend radiologisch onderzoek voor de klinische diagnose bij individuele patiënten en de phenotypering ter voorbereiding op moleculair genetische analyse.

Het MCPP-profiel, zoals wij dat vonden bij BHMED, was significant anders dan dat van EDM2 en was consistent met de klinische diagnose, de radiologische waarnemingen en de segregatie van de matrilin-3 mutatie (Hoofdstuk 4.4). Door de MCPP profile analysis konden we aantonen, dat een gedisproportionaliseerd verkorte middenphalanx van digitum 2 manus (MPh2) bij zowel aangedane als

niet-aangedane BHMED familieleden aanwezig was, en welke derhalve los staat van de aandoening BHMED. Deze studie toonde de grote waarde van de MCPP-profiel analyse voor phenotypische classificatie bij onderzoek van grotere families met veel aangedane personen. De differentiaal diagnostische waarde bij patiënten die niet in samenhang met familieleden kunnen worden onderzocht, was geen specifiek onderwerp van dit onderzoek.

Hoofdstuk 5 bevat een uitgebreide discussie van de resultaten van deze dissertatie en een aantal kanttekeningen ten aanzien van toekomstig wetenschappelijk werk. De vier doelen van deze studie, verdere klinische beschrijving van het BHMED-phenotype, radiologische karakterisering, identificatie van de moleculair genetische basis, en het bevestigen van de aanvullende waarde van de MCPP profile analysis met gebruik van het genotype als gouden standaard, werden alle bereikt. Deze studie toonde ook de mogelijk belangrijke rol van de door 'height-related partner choice' beïnvloede polygenetische achtergrond tegenover de matrilin-3 gen effecten ten aanzien van totale lichaamslengte. Onze waarnemingen kunnen wellicht een bijdrage leveren aan het ontwikkelen van modellen waarmee de afzonderlijke bijdragen en interacties van geneffecten op kwantitatieve kenmerken worden gemeten.

De identificatie van genen die betrokken zijn bij skeletdysplasieën en predisponeren voor premature osteoarthropathieën, kan ook bijdragen tot een beter inzicht in de complexe ontwikkeling en moleculaire basis van deze aandoeningen, voor het ontwikkelen van nieuwe behandelingsmethoden, tot de keuze van de beste behandeling in geval van vergelijkbare klinische condities met toch verschillende onderliggende defecten, tot het identificeren van exogene risicofactoren die een aangrijpingspunt voor preventieve maatregelen kunnen opleveren, en tot het verschaffen van een goede basis voor genetische counselling.

CHAPTER 7

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B.R.H. Jansen, MD, PhD, dear co-promoter and supervisor, dear doctor Jansen, after to-day perhaps: dear Bernard (?). Thanks to your inspiration and supervision, this work could be undertaken and completed. In times of doubt, there was yet another supportive telephone call, sometimes even in the early hours of the night. Whenever progress tended to slow down, we put our heads together again. A second baby was welcome only when the second article was finished. The third one after the third article and so on. At the time, our interests ran parallel. Many thanks for your encouragement with the multiplicity of sayings from a wide range of backgrounds.

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Professor J.R. van Horn, esteemed promoter, dear teacher, dear Jim. From the beginning of the study you have been involved in designing it. You have always stimulated me to finish the thesis during the training for an orthopaedic surgeon. When this misfired, you even thought that the cigar may go out. But you see, just a little puff and the tobacco starts to glow again. Jim, after an eventful period in the field of health, I am glad that together we have finalised the work.

P.F. Dijkstra, MD, PhD, highly valued as a colleague but rather as a human being. Unfortunately, you are unable to join in the completion of our scientific work. This summer we received the tragic news. Initially, you were an essential

link in the radiological confirmation of being affected with BHMED or not. Without your help, our latest article would never have become what it is now.

E. Wesby-van Swaay, MD, PhD, dear Eveline. When we had mapped the BHMED family initially, we got into contact with clinical genetics in Rotterdam, which led us to you. Your comments on the physical examination, which in clinical-genetic terms is more comprehensive than its orthopaedic counterpart, were very useful. We re-examined many relatives, finding abnormalities, including those of the hands. We are still in touch, even though you are far away in Helsinki, Finland. Fare you well.

P. Heutink, MD, PhD, dear Peter. In spite of your many current research lines in clinical genetics, you have been of great value for the practical characterisation of the BHMED genome. How pleased we were when your E-mail revealed that the gene defect had been found. Peter, many thanks and I wish you good luck in your future steps forward.

N.J.G.M. Veeger, dear Nic. During my academic year, we have racked our brains for many an hour over the MCPP data. My special thanks are due to you for your value as a statistician, who has taught me its principles.

Laurieke van Kammen, dear Laurieke, you were the central figure in the first clinical phase at Delft, where I was given the opportunity to see the many relatives in the evening and where you succeeded in tracing (their) X-rays from all over the country in an excellent way. Many thanks to you.

Jolanda Hoekstra, "my" secretary. Jolanda, you did one thing my wife Cora is not allowed to do at home: clearing my desk. When it – frequently – came to pass that I had a mere 10 square centimeters to write on at my desk, you were the one to multiply it by a factor 10. I am sorry you are going to leave us.

Linda Offermans, Professor Lindhout's secretary at Utrecht, a special word for you. Especially in the final phase of the completion of the doctoral thesis, you were

a pivot in its finishing touch. You were most accurate in dealing with appointments concerning its completion in Dick's overfull agenda. You always managed to fit me in. Thanks for your effort. You are a cracker!

Jeannette Lokker, Professor Lindhout's secretary at Rotterdam, you too deserve a word of thanks. Your handling of many appointments in the early phase was flawless. Even after Dick had left for Utrecht, I could still count on you. Thank you!

Rein van der Wal, hospital photographer at Zwolle. Rein, with your help the many X-rays could be digitalised at a qualitatively very high level, of which we gratefully made use. I hope our cooperation will continue for a long time to come. Thanks!

Professor P. Nikkels, dear Peter. Thank you for the histological tests that were performed with the specimens of arthrotic joints. Hopefully, we may continue to build on the data we now have in a later stage.

Orthopaedic personnel of the Isala Clinics, location Weezenlanden, Zwolle. Dear colleagues, dear Robert, René, George, Niek and Kees, Thank you for the time I was given to devote myself to this study. You have also trained a doctor who may join your ranks. Actually, the nameplate on the door: Dr. A.K. Mostert should not be there until today!

Orthopaedic personnel and assistants of Groningen University Hospital. Dear colleagues, I thank you too for the rare hours I was permitted to spend "during the boss's time" in the room where we were on duty and which still smelled of cigars months after I had left.

J. van Deursen, MD, famous general practitioner at Zutphen, dear Jos. A special word of thanks to you. During my final years as a radiographer, you assured me that the study of medicine would not be aiming too high. You encouraged me to

suit the action to the word. That is the reason why today I wanted to have you by my side during this ceremony.

M.M.F.M. Bressers, MD, valued colleague, dear Mart. During our orthopaedic training programme, and even before, I got to know you as a talented colleague, whom I could always rely on. For you were always around to help. This then is the reason that today I wanted to have you by my side during this ceremony.

Harmen Ettema, promising resident orthopaedic surgeon. Thanks for helping me in the final phase of writing this dissertation, especially when the computer crashed and time goes on. Even in the late evening, you were available to give assistance.

Fred Mostert, MA, sworn translator, distant uncle, I thank you too for correcting the English text in the final phase. You were ever accessible.

Thea Schenk, very many thanks for your assistance to make the 'booklet' ready for printing in the final phase.

Members of the examined family and in particular those that have given their assistance in contacting other familiy members. Many thanks for cooperating in this study of which the results are presented in this thesis. Your contribution to this study enabled a better understanding of the nature and heridity of this disease.

L. Elsbach, MD, late fellow orthopaedic surgeon at Delft, initiator of our study. We also wish to mention him as our study is based on a publication of his in the *Journal of Bone and Joint Surgery [B]* of 1959.

Cora, my darling, what a liberty you have given me to complete this thesis in addition to the study and the work but most of all in addition to the growing family! The booklet is finished! Thank you for your support, love and patience.

My parents, dear mum and dad, I am delighted that you too are present at this event. Your interest in the study has always been a stimulus to proceed. Thanks for who you were and are!

In imitation of the conclusion of the inaugural speech by the professor of rheumatology in the orthopaedic surgery, Professor Pöll, lately appointed, I conclude with his words that I endorse:

Sit nomen Domini benedictum.

ACKNOWLEDGEMENTS

NEDERLANDS

Dr. B.R.H. Jansen, waarde co-promotor, geachte opleider, beste doctor Jansen, na vandaag misschien: beste Bernard (?). Door uw inspiratie en begeleiding kon deze promotie worden gestart en afgerond. In momenten van twijfel kwam daar weer een doortastend telefoontje, soms zelfs in de vroege nacht. Als de voortgang enigszins stagneerde, werden de koppen weer bij elkaar gestoken. Er mocht pas een tweede kindje komen, wanneer het tweede artikel klaar zou zijn. Een derde na het derde enzovoorts. Indertijd waren deze belangen wederzijds in elkaar verstrengeld. Veel dank voor het bemoedigen met de veelheid van teksten uit allerlei achtergronden!

Prof. Dr. D. Lindhout, hooggeleerde promotor, beste Dick, met veel plezier terugdenkend aan de vele uurtjes op je zolderkamer in Leiden, die zo vruchtbaar bleken te zijn, maar mij maar niet snel genoeg gingen. De heerlijke (Deense) lunches, door Lisbeth je vrouw geserveerd, zal ik niet snel vergeten. Je accuratesse heeft zijn vruchten afgeworpen, ook al gaat dat vaak voor snijdend specialisten trager dan gewenst. Dick, veel dank voor je prikkelende doorzettingsvermogen.

Prof. Dr. J.R. van Horn, hooggeleerde promotor, geachte opleider, beste Jim. Vanaf het begin van het onderzoek ben jij betrokken geweest bij de opzet van de studie. Jij hebt er steeds op aan gestuurd tijdens de opleiding tot orthopaedisch chirurg tot een afronding te komen van de dissertatie. Toen dat net niet lukte, had jij even het idee, dat de sigaar zou doven. Maar zie hier, even trekken en de tabak gaat weer gloeien. Jim, na een bewogen episode op gezondheidsgebied, ben ik blij, dat wij samen tot een goede afronding hebben kunnen komen.

Dr. P.F. Dijkstra, zeer gewaardeerde collega en nog meer als mens. Helaas mag jij deze afronding van ons wetenschappelijk werk niet meer meemaken. De afgelopen zomer bereikte ons dit tragische bericht. Jij bent initieel een essentiële

schakel geweest in de radiologische bevestiging van het al dan niet zijn aangedaan met BHMED. Ook ons laatste artikel was zonder jouw hulp nooit dat geworden, wat het nu is.

Dr. E. Wesby-van Swaay, beste Eveline. Toen we aanvankelijk de BHMED-familie in kaart hadden gebracht, zochten we contact met de klinische genetica in Rotterdam en kwamen bij jou terecht. Heel waardevol waren je opmerkingen ten aanzien van het lichamelijk onderzoek, dat klinisch genetisch iets uitgebreider is dan het orthopaedische. Vele familieleden hebben we wederom onderzocht en vonden afwijkingen, waaronder ook die van de handen. Zelfs nu je in Helsinki, Finland, zit, hebben we nog contact. Het ga je goed.

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Jolanda Hoekstra, "mijn" secretaresse. Jolanda, jij deed één ding, wat mijn vrouw Cora thuis niet mag doen: mijn bureau opruimen. Wanneer ik frequent nog maar 10 cm^2 bureauruimte om te schrijven had, was jij degene, die daar een factor 10 bijtoverde. Jammer dat je ons gaat verlaten.

Linda Offermans, secretaresse van Professor Lindhout in Utrecht, een speciaal woord voor jou. Jij was met name in de laatste fase van de afronding een centrale spil rondom de afwerking van de dissertatie. Zeer accuraat ging je om met afspraken rondom de afronding in de overvolle agenda van Dick. Altijd was er een gaatje te vinden. Dank voor je inzet. Je bent een topper!

Jeannette Lokker, secretaresse van Professor Lindhout in Rotterdam, ook jij verdient een woord van dank. Vele afspraken in de beginfase verliepen via jou feilloos. Ook na het vertrek van Dick naar Utrecht, kon ik nog steeds op je rekenen. Dank!

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Orthopaedische staf van de Isala Klinieken, locatie Weezenlanden, **Zwolle**. Geachte collegae, beste Robert, René, George, Niek en Kees. Dank voor de tijd die ik kreeg, om aan deze studie te werken. Jullie hebben eveneens een doctor opgeleid, die jullie gelederen mag komen versterken. Het bordje op de deur: Dr. A.K. Mostert mag er eigenlijk pas vanaf vandaag op hangen!

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Dr. L. Elsbach, wijlen collega orthopaedisch chirurg te Delft, initiator van ons onderzoek. We willen hem ook noemen, omdat ons onderzoek is gebaseerd op een publicatie van zijn hand in de *Journal of Bone and Joint Surgery [B]* van 1959.

Cora, lieve schat, wat heb jij mij een vrijheid gegeven om naast de studie en het werk, maar bovenal naast het opgroeiende gezin, deze promotie af te ronden! Het boekje is af! Dank voor je steun, liefde en geduld.

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In navolging van de afsluiting van de inaugurele rede van de laatst aangestelde hoogleraar rheumatologie in de orthopaedie, Professor Pöll, sluit ik af met zijn woorden, die ik onderschrijf:

Sit nomen Domini benedictum.

CHAPTER 8

CURRICULUM VITAE

The author of this thesis, Adrianus Klazinus Mostert, was born on April 30, 1962 at Winschoten, the Netherlands, as the son of a clergyman. After obtaining his secondary school certificate at the Willem van Oranje College at Waalwijk, he spent his compulsory military service in the Royal Dutch Navy, on board HrMs Zuiderkruis. From 1982 to 1989, he worked as a radiographer in the X-ray department at Leiderdorp and Harderwijk as well as in radiotherapy at Deventer and The Hague.

From 1987 to 1989, he studied for his pre-university certificate during evening classes. Then he started the study of medicine at the University of Amsterdam in the same year, doing experimental surgery to detect pseudoarthroses in animal models at the Amsterdam University teaching hospital (head Professor P.J. Klopper). The initial degree programme was finished in three years in 1992, and the internship was done at the State University of Leiden until 1994.

He worked as a resident orthopaedic surgeon at Delft (Reinier de Graaf Hospital, head B.R.H. Jansen, MD, PhD) and at Zwolle (Weezenlanden Hospital, head R.M. Castelein, MD, PhD) until 1995.

In January 1996, he started as a resident general surgeon at the Reinier de Graaf Infirmary at Delft (head P. de Graaf, MD, PhD), and January 1998 saw him as a resident orthopaedic surgeon in the Isala Clinics, location Weezenlanden at Zwolle (head R.M. Castelein, MD, PhD). In 2000 he did his academic year at Groningen University Hospital (head Professor J.R. van Horn).

In 1999 he was further trained at Stolzalpe, Austria, by professor R. Graf in ultrasonound examination of neonatal hip development, followed by a fellowship

in traumatology at the University Hospital of Ghent, Belgium (head Professor R. Verdonk).

Since January 1, 2002, he has been an orthopaedic surgeon. From May 1, 2002, he has been associated with the orthopaedic surgeons Robert Bots, Niek Tulp and Kees Verheijen and general surgeons Adrie Blomme, André Burghard, Wilbert Fritschy, Maarten Lopes Cardozo, Robert Pierik in the Isala Clinics, location Weezenlanden, at Zwolle.

He is married to Cora Mostert-Deelen, and presently they have three children: Jan-Hans, Pauline and Martijn.

CHAPTER 9

PUBLICATIONS

PUBLICATION LIST

Ankle stability without the lateral malleolus – report of two cases.
Mostert AK, Marijnissen WJ, Bongers KJ, Jansen BRH.
Acta Orthop Scand 1996;67(6):622–623.

Laterale malleolus: altijd nodig? Casuïstiek.
Mostert AK, Marijnissen WJ, Bongers KJ, Jansen BRH.
Ned Tijdschr Geneeskd 1996;140(8):461.

Tuberculose als onverwachte oorzaak van een mammatumor.
Mostert AK, Nijhuis-Heddes JMA, Sastrowijoto SH, Persijn NJM.
Med J Delft 1997;4:197–199.

Ankle stability without the lateral malleolus – report of two cases.
Mostert AK, Marijnissen WJ, Bongers KJ, Jansen BRH.
Foot & Ankle Int 1997;18(7):454.

Laterale malleolus: altijd nodig? Casuïstiek.
Mostert AK, Marijnissen WJ, Bongers KJ, Jansen BRH.
Med J Delft 1997;4:218–221.

Echografische screening van kinderheupen (het SOUNDCHECK project).
Mostert AK, Boere-Boonekamp MM, Geertsma TSA, Castelein RM.
Ned Tijdschr Orthop 1999;2:59–60.

Verslag Fellowship Traumatologie Universitair Ziekenhuis Gent.
Mostert AK.
Ned Tijdschr Orthop 1999;4:40–41.

Commentaren op De Nies, Custers en Willems (NTvO 1999;2:27–30).
Mostert AK, Castelein RM.
Ned Tijdschr Orthop 1999;4:34.

De Pavlik-bandage bij de repositiebehandeling van een heupluxatie.
Mostert AK, Tulp NJA, Castelein RM.
Ned Tijdschr Orthop 1999;2:43.

De Pavlik-bandage bij de repositiebehandeling van een heupluxatie.
Mostert AK, Tulp NJA, Castelein RM.
Ned Tijdschr Geneeskd 1999;143(30):1589–1590.

Results of Pavlik Harness Treatment for neonatal Hip Dislocation as related to Graf's Sonographic Classification.
Mostert AK, Tulp NJA, Castelein RM.
J Pediatr Orthop 2000;20(3):306–310.

Results of Pavlik Harness Treatment for neonatal Hip Dislocation as related to Graf's Sonographic Classification.
Mostert AK, Tulp NJA, Castelein RM.
Ned Tijdschr Geneeskd 2000;144(32):1553.

De dubbelzijdige idiopathische patellapeesruptuur.
Mostert AK, te Slaa RL.
Ned Tijdschr Orthop 2000;2:12–15.

De dubbelzijdige idiopathische patellapeesruptuur – Sporttraumatologische Casuïstiek.
Mostert AK, Marijnissen WJ, Bongers KJ, Jansen BRH.
NOV-boekje 2000;91–93.

Tuberculose als onverwachte oorzaak van een mammatumor.
Mostert AK, Nijhuis-Heddes JMA, Sastrowijoto SH, Persijn NJM.
Tegen de Tuberculose (KNCV) 2000;2:51–53.

Ongebruikelijke presentatie van calcaneusfracturen.
Mostert AK, Lamoré JJP, Verburg H, Koning J.
Ned Tijdschr Orthop 2000;1:28–32.

Reaktie op commentaar op Ongebruikelijke presentatie van calcaneusfracuren.
Mostert AK, Louwerens JWK.
Ned Tijdschr Orthop 2000;3:47–48.

Closed intramedullary tibial nailing using the Marchetti-Vicenzi nail.
DeSmet K, Mostert AK, DeWitte J, DeBrauwer V, Verdonk R.
Injury 2000;31:597–603.

Closed intramedullary tibial nailing using the Marchetti-Vicenzi nail.
DeSmet K, Mostert AK, DeWitte J, DeBrauwer V, Verdonk R.
Ned Tijdschr Orthop 2000;3:35.

Verslag van het 6th International Symposium on Clinical Disorders of Bone and Mineral Metabolism (formerly Henry Ford Symposium) in Venetië.
Mostert AK.
Ned Tijdschr Orthop 2000;2:52–53.

Orthokwis: Door het oog van de naald.
Mostert AK, Schenk W.
Ned Tijdschr Orthop 2000;4:31–34.

Tot op het bot betrokken, BHMED.
Bijdrage boekwerk bij het afscheid van Dr. B.R.H. Jansen.
Mostert AK.
2000; ISBN No. 90-9014510-9.

Letter to the Editor: Pavlik Harness.
Mostert AK, Tulp NJA, Castelein RM.
J Pediatr Orthop [B] 2002;11:181.

Letter to the Editor: Pavlik Harness.
Mostert AK, Tulp NJA, Castelein RM.
J Pediatr Orthop [A] 2002;22:410.

Osteochondritis dissecans of the femoral head in Perthes' disease. A case report.
Mostert AK, Bots RAA.
(Submitted)

Pelvic and femur fracture at cesarean section. A case report.
Mostert AK, Heeg M.
(Submitted)

Congenital synostosis of the proximal forearm. A case report.
Mostert AK, Tulp NJA.
Curr Orthop 2002;16(5)395–397.

Bilateral hereditary micro-epiphyseal dysplasia: Further delineation of the phenotype with 40 years follow-up.
Mostert AK, Jansen BRH, Dijkstra PF, Wesby-van Swaay E, van Horn JR, Heutink P, Lindhout D.
Int Orthop 2002;26(3):188–193.

Radiological features of bilateral hereditary micro-epiphyseal dysplasia. - A distinct entity in the skeletal dysplasias – [Radiologische Besonderheiten einer bilateral vererblichen Mikro-Epiphysendysplasie – deutliche Entität einer Skelettdysplasie].
Mostert AK, Dijkstra PF, van Horn JR, Jansen BRH, Heutink P, Lindhout D.
RöFo Fortschr Geb Röntgenstr Neuen Bildgeb Verfahr 2002;174(7):887–892.

PUBLICATIONS

Familial multiple epiphyseal dysplasia due to a matrilin-3 mutation. Further delineation of the phenotype including 40 years follow-up.
Mostert AK, Dijkstra PF, Jansen BRH, van Horn JR, de Graaf B, Heutink P, Lindhout D.
Am J Med Genet 2003;in press.

Metacarpophalangeal pattern (MCPP) profile analysis in a Dutch family with bilateral hereditary micro-epiphyseal dysplasia linked to matrilin-3.
Mostert AK, Dijkstra PF, Jansen BRH, Veeger NJGM, van Horn JR, de Graaf B, Heutink P, Lindhout D.
(Submitted)

POSTER PRESENTATIONS

A large family with Bilateral Hereditary Micro-Epiphyseal Dysplasia.
Mostert AK, Jansen BRH, Dijkstra PF, van Horn JR, Lindhout D.
Symposium Clinical Disorders of Bone and Mineral Metabolism.
Venice, Italy, 20–25 November 1999.

A large family with Bilateral Hereditary Micro-Epiphyseal Dysplasia.
Mostert AK, Jansen BRH, Dijkstra PF, van Horn JR, Lindhout D.
12th SICOT European Trainees Meeting,
Paris, France, 30–31 August 2001.
WINNING POSTER AWARD PRESENTATION.

APPENDIX 1

Figure 1. Arthrogram of the right hip of an 8-year-old boy with BHMED (IV-26), showing the marked deformity of the small bony epiphysis, especially on the medial side, and a normal round cartilage cover. The metaphysis is unaffected and the acetabulum is normally shaped.

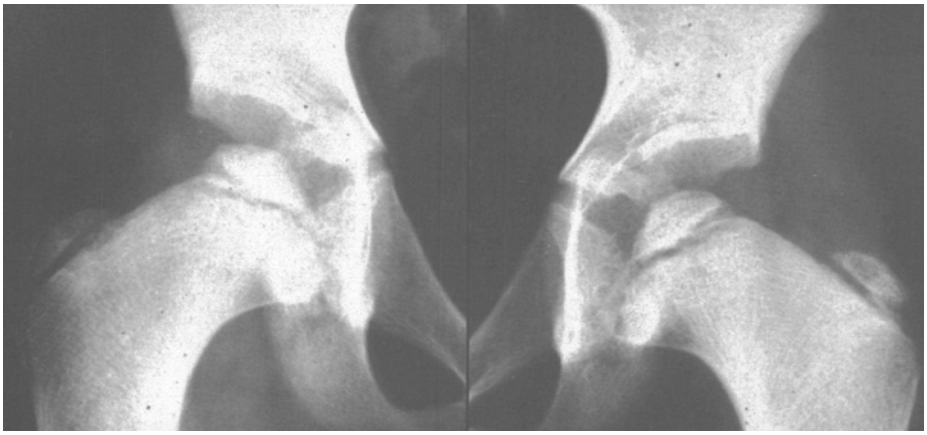

Figure 2. The X-ray of the both hips of the same 8-year-old boy, showing the small bony epiphyses.

Figure 3. The X-ray of the pelvis of the same affected BHMED man at the age of 37 with a total hip arthroplasty on the left side and the marked deformity on the right side: flattening of the epiphysis, a short and thickened collumn and evident signes of osteoarthritis.

These are the only radiographs we could overtake of the history archives of BHMED family members. Unfortunately, the rest of the radiographs were annihilated due to storage problems.

APPENDIX 2

INTERNATIONAL NOMENCLATURE OF CONSTITUTIONAL DISORDERS OF BONE OSTEOCHONDRODYSPLASIAS

SOURCE: http://www.csmc.edu/genetics/skeletdys/
(Version December 2002)

	Mode of Inher-itance	*OMIM Syn-drome*	*Pre-sent at Birth*	*Chrom-osomal Locus*	*Gene*	*Protein*	*OMIM Gene/ Protein*
1. Achondroplasia group							
Thanatophoric dysplasia, Type I	AD	187600	+	4p16.3	FGFR3	FGFR3	134934
Thanatophoric dysplasia, Type II	AD	187610	+	4p16.3	FGFR3	FGFR3	134934
Achondroplasia	AD	100800	+	4p16.3	FGFR3	FGFR3	134934
Hypochondroplasia	AD	146000	–	4p16.3	FGFR3	FGFR3	134934
Other FGFR3 disorders							
2. Spondylodysplastic and other perinatally lethal groups							
Lethal platyspondylic skeletal dysplasias	SP	270230	+				
(San Diego type, Torrance type, Luton type)		151210	+				
Achondrogenesis type 1A	AR	200600	+				
3. Metatropic dysplasia group							
Fibrochondrogenesis	AR	228520	+				
Schneckenbecken dysplasia	AR	269250	+				
Metatropic dysplasia (various forms)	AD	156530	+				
4. Short-rib dysplasia (SRP) (with or without polydactyly) group							
SRP type I, Saldino-Noonan	AR	263530	+				
SRP type II, Majewski	AR	263520	+				
SRP type III, Verma-Naumoff	AR	263510	+				
SRP type IV, Beemer-Langer	AR	269860	+				
Asphyxiating thoracic dysplasia (Jeune)	AR	208500	+				
Chondroectodermal Dyplasia (Ellis-van Creveld dysplasia)	AR	225500	+	4p16			
5. Atelosteogenesis-omodysplasia group							
Atelosteogenesis type I (includes 'Boomerang dysplasia')	SP	108720	+				

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
Omodysplasia I (Maroteaux)	AD	164745	+				
Omodysplasia II (Borochowitz)	AR	258315	+				
Otopalatodigital syndrome type II	XLR	304120	+				
Atelosteogenesis Type III	SP	108721	+				
de la Chapelle dysplasia	AR	256050	+				
6. Diastrophic dysplasia group							
Diastrophic dysplasia	AR	222600	+	5q32-q33	DTDST	Sul. Transporter	
Achondrogenesis 1B	AR	600972	+	5q32-q33	DTDST	Sul. Transporter	
Atelosteogenesis type II	AR	256050	+	5q32-q33	DTDST	Sul. Transporter	
7. Dyssegmental dysplasia group							
Dyssegmental dysplasia, Silverman-Handmaker type	AR	224410	+				
Dyssegmental dysplasia, Rolland-Desbuquois type	AR	224400	+				
8. Type II collagenopathies							
Achondrogenesis II (Langer-Saldino)	AD	200610	+	12q13.1-q13.3	COL2A1	Type II collagen	120140
Hypochondrogenesis	AD	200610	+	12q13.1-q13.3	COL2A1	Type II collagen	120140
Kniest dysplasia	AD	156550	+	12q13.1-q13.3	COL2A1	Type II collagen	120140
Spondyloepiphyseal dysplasia (SED) congenital	AD	183900	+	12q13.1-q13.3	COL2A1	Type II collagen	120140
Spondyloepimetaphyseal dysplasia (SEMD) Strudwick type	AD	184250	+	12q13.1-q13.3	COL2A1	Type II collagen	120140
SED with brachydactyly	AD			12q13.1-q13.3	COL2A1	Type II collagen	120140
Mild SED with premature onset arthrosis	AD		–	12q13.1-q13.3	COL2A1	Type II collagen	120140
Stickler dysplasia (heterogeneous, some not linked to COL2A1)	AD	108300	+	12q13.1-q13.3	COL2A1	Type II collagen	120140

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
9. Type XI collagenopathies							
Stickler dysplasia (heterogeneous)	AD	184840	+	6p21	COL11A1	Type XI collagen	120280
	AR	215150	+	6p21.30	COL11A2	Type XI collagen	120290
Otospondylomegaepiphyseal dysplasia (OSMED)	AD		+	6p21.3	COL11A2	Type XI collagen	120290
10. Other spondyloepi-(meta)-physeal [SE(M)D] dysplasias							
X-linked spondyloepiphyseal dysplasia tarda	XLD	313400	–	Xp22.2-p22.1			
Other late-onset spondyloepi(meta)physeal dysplasias (Irapa) (Namaqualand et al.)	AR	271650	–				
Progressive pseudorheumatoid dysplasia	AR	208230	–				
Dyggve-Melchior-Clausen dysplasia	AR	223800	+				
Wolcott-Rallison dysplasia	AR	226980	–				
Immuno-osseous dysplasia-Schimke	AR	242900	+				
Opsismodysplasia	AR	258480	+				
Chondrodystrophic myotonia	AR	258480	+				
(Schwartz Jampel), type 1, type 2	AR	255800	+	1q36-34			
Spondyloepiphyseal dysplasia with joint laxity	AR	271640	+				
Sponastrime dysplasia	AR	271510	–				
SEMD short limb – abnormal calcification	AR	271665	+				
11. Multiple epiphyseal dysplasias & pseudoachondroplasia							
Pseudoachondroplasia	AD	177170	–	19p12-13.1	COMP	COMP	600310
Multiple epiphyseal dysplasia (MED)	AD	132400	–				
(Fairbanks and Ribbing types)	AD	600204	–	19p12-13.1	COMP	COMP	600310
Other MEDs ?	?	600969	–	1p32.2-33	COL9A2	Type IX collagen	120260

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
12. Chondrodysplasia punctata (stippled epiphyses group)							
Rhizomelic type	AR	215100	+	4p16-p14	PEX7	peroxin-7	601757
Zellweger syndrome	AR	214100	+	7q11.23	PEX1		
	AR	214100	+	6p21.1	PEX6	peroxin-6	601498
	AR	214100	+	7q11.23	PEX1	peroxin-1	602136
	AR	214100	+	12	PEX5	peroxin-5	
	AR	214100	+	8q21.1	PEX2	peroxin-2	170993
Conradi-Hünermann type	XLD	302950	+	Xq28	CPXD		
X-linked recessive type	XLR	302940	+	Xp22.3	CPXR		
Brachytelephalangic type	XLR	302940	+	Xp22.32	ARSE	aryl-sulfatase	E 302950
Tibial-metacarpal type	AD	118651	+				
Vitamin K-dependent coagulation defect	AR	277450	+				
Other acquired and genetic disorders including Warfarin embryopathy							
13. Metaphyseal dysplasias							
Jansen type	AD	156400	+	3p22-p21.1	PTHR	PTHR/PTHRP	168468
Schmid type	AD	156500	–	6q21-q22.3	COL10A1	COL10 α chain	120110
McKusick type (cartilage-hair hypoplasia)	AR	250250	+	9p13			
Metaphyseal anadysplasia	XLR?	309645	–				
Metaphyseal dysplasia with pancreatic insufficiency and cyclic neutropenia (Shwachman Diamond)	AR	260400	–				
Adenosine deaminase deficiency	AD	102700	–	20q-13.11	ADA	Adenosine deaminase	102700
Metaphyseal chondrodysplasia-Spahr type	AR	250400	–				
Acroscyphodysplasia (various types)	AR	250215	–				
14. Spondylometaphyseal dysplasias (SMD)							
Spondylometaphyseal dysplasia Kozlowski type	AD	184252	+				
Spondylometaphyseal dysplasia (Sutcliffe type)	AD	184255	+				
SMD with severe genu valgum (includes Schmidt and Algerian types)	AD	184253	+				

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
SMD Sedaghatian type	AR		+				
Mild SMD different types that have not been well delineated			–				
15. Brachyolmia spondylodysplasias							
Hobaek (includes Toledo type)	AR	271530-630	–				
Maroteaux type	AR	–					
Autosomal dominant type	AD	113500	–				
16. Mesomelic dysplasias							
Dychondrosteosis (Leri-Weill)	AD	127300	–				
Langer type (homozygous dyschondrosteosis)	AR	249700	+				
Nievergelt type	AD	163400	+				
Kozlowski-Reardon type	AR		+				
Reinhardt-Pfeiffer type	AD	191400	+				
Werner type	AD		+				
Robinow type, dominant	AD	180700	–				
Robinow type, recessive	AR	268310	–				
Mesomelic dysplasia with synostoses	AD	600383	+				
17. Acromelic and acromesomelic dysplasias							
Acromicric dysplasia	AD	102370	+				
Geleophysic dysplasia	AR	231050	+				
Weill-Marchesani dysplasia	AR	277600	+				
Cranioectodermal dysplasia	AR	218330	+				
Trichorhinophalangeal dysplasia, type I	AD	190350	+	8q24.12	TRPS1		
Trichorhinophalangeal dysplasia, type II (Langer-Giedeon)	AD	150230	+	8q24.11 -q24.13	TRPS1+ EXT1		
Trichorhinophalangeal dysplasia, type III	AD	190351	+				
Grebe dysplasia	AR	200700	+	20q11.2	CDMP1	cartilage derived morphogenic protein 1	601146
Hunter-Thompson dysplasia	AR	201250	+	20q11.2	CDMP1	cartilage derived morphogenic protein 1	601146

(Continued from previous page)	***Mode of Inheritance***	***OMIM Syndrome***	***Present at Birth***	***Chromosomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
Brachydactyly type A1-A4	AD	112500-800	+				
Brachydactyly type B	AD	113000	+				
Brachydactyly type C	AD	133100	+	21q11	CDMP1	cartilage derived morphogenic protein 1	601196
	AD			12q24			
Brachydactyly type D	AD	113200	+				
Brachydactyly type E	AD	113000	–				
Pseudohypopara-thyroidism (Albright Hereditary Osteodystrophy), various types, see OMIM				20q13	GNAS1	quanine nucleotide binding protein of edenylate cyclase α-subunit	139320
Acrodysostosis	SP (AD)	101800	–				
Saldino-Mainzer dysplasia	AR	266920	–				
Brachydactyly-hypertension dysplasia (Bilginturan)	AD	112410	+	12p			
Craniofacial conodysplasia	AD		+				
Angel-shaped phalango-epiphyseal dysplasia (ASPED)	AD	105835	+				
Acromesomelic dysplasia	AR	201250	+				
Other acromesomelic dysplasias							
18. Dysplasias with prominent membranous bone involvement							
Cleidocranial dysplasia	AD	119600	+	6p21	CBFA1	core binding factor α1-subunit	600211
Osteodysplasty, Melnick-Needles	XLD	309350	–				
Precocious osteodysplasty (terHaar dysplasia)	AR		+				
Yunis-Varon dysplasia	AR	216340	+				
19. Bent-bone dysplasia group							
Campomelic dysplasia	AD	114290	+	7q24.3-q25.1	SOX9	SRY-box 9	211970

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
Kyphomelic dysplasia	?AR	211350	+				
Stüve-Wiedemann dysplasia	AR	601559	+				
20. Multiple dislocations with dysplasias							
Larsen syndrome	AD	150250	+	3p21.1-p14.1	LARI		
Larsen-like syndromes (including La Reunion Island)	AR	245600	+				
Desbuquois dysplasia	AR	251450	+				
Pseudodiastrophic dysplasia	AR	264180	+				
21. Dysostosis multiplex group							
Mucopolysaccharidosis IH	AR	252800	–	4p16.3	IDA	α-1-Iduronid-ase	
Mucopolysaccharidosis IS	AR	252800	–	4p16.3	IDA	α-1-Iduronid-ase	
Mucopolysaccharidosis II	XLR	309900	–	Xq27.3-q28	IDS	Iduronate-2-sulfatase	
Mucopolysaccharidosis IIIA	AR	252900	–	17q25.3	HSS	Heparan sulfate sulfatase	
Mucopolysaccharidosis IIIB	AR	252920		17q21		N-Ac-α-D-glucos-aminidase	
Mucopolysaccharidosis IIIC	AR	252930	–			Ac-CoA: α-glucos-aminidase-N-acetyl-transferase	
Mucopolysaccharidosis IIID	AR	252940	–	12q14	GNS	N-Ac-glucos-amine-6-sulfatase	
Mucopolysaccharidosis IVA	AR	230500	–	16q24.3	GALNS	Galactose-6-sulfatase	
Mucopolysaccharidosis IVB	AR	230500	–	3p21.33	GLBI	β-Galactosid-ase	
Mucopolysaccharidosis VI	AR	253200	–	5q13.3	ARSB	Arylsulfat ase B	

(Continued from previous page)	***Mode of Inheritance***	***OMIM Syndrome***	***Present at Birth***	***Chromosomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
Mucopolysaccharidosis VII	AR	253200	–	7q21.11	GUSB	β-Glucuronidase	
Fucosidosis	AR	230000	–	1p34	FUCA	α-Fucosidase	
- Mannosidosis	AR	248500	–	19p13.2-q12	MAN	α-Mannosidase	
- Mannosidosis	AR	248510	–	4	MANB	β-Mannosidase	
Aspartylglucosaminuria	AR	208400	–	4q23-q27	AgA	Aspartylglucosaminidase	
GM1 Gangliosidosis, several forms	AR	230500	+	3p21-p14.2	GLB1	β-Galactosidase	
Sialidosis, several forms	AR	256550	+/–	6p21.3	NEU	α-Neuraminidase	
Sialic acid storage disease	AR	269920	+/–	6q14-q15	SIASD		
Galactosialidosis, several forms	AR	256540		20q13.1	PPGB	β-Galactosidase protective protein	
Multiple sulfatase deficiency	AR	272200	+/–			Multiple sulfatases	
Mucolipidosis II	AR	252500	+	4q21-23	GNPTA	N-Ac-Glucosamine phosphotransferase	
Mucolipidosis III	AR	252600	–	4q21-23	GNPTA	N-Ac-Glucosamine phosphotansferase	
22. Osteodysplastic slender bone group							
Type I osteodysplastic dysplasia	AR	210710	+				
Type II osteodysplastic dysplasia	AR	210720	+				
Microcephalic osteodysplastic dys.	AR						
23. Dysplasias with decreased bone density							
Osteogenesis imperfecta I (without opalescent teeth)	AD	166200	+/–	17q21	COL1A1	α(1) I procollagen	120150
Osteogenesis imperfecta I (with opalescent teeth)	AD	166240	+/–	17q21	COL1A1	α(1)I procollagen	120150

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
	AD	166240	+/-	7q22.1	COL1A2	α(2)I pro-collagen	120160
Osteogenesis imperfecta II	AD	166210	+	17q21 COL1A 1	α(1)I procollag en	120150	
	AD	166210	+	7q22.1	COL1A2	α(2)I pro-collagen	120160
	AR	259400	+	17q21	COL1A1	α(1)I pro-collagen	120150
Osteogenesis imperfecta III	AD	259420	+	17q21	COL1A1	α(1)I pro-collagen	120150
	AD	259420	+	7q22.1	COL1A2	α(2)I pro-collagen	120160
	AR	259420	+	7q22.1	CO L1A2	α(2)I pro-collagen	120160
	AR	259420	+				
Osteogenesis imperfecta IV (without opalescent teeth)	AD	166220	+	7q22.1	COL1A2	α(2)I pro-collagen	120160
	AD	166220	+	17q21	COL1A1	α(1)I pro-collagen	120150
Osteogenesis imperfecta IV (with opalescent teeth)	AD	166220	+	7q22.1	COL1A2	α(2)I pro-collagen	120160
	AD	166220	+	17q21	COL1A1	α(1)I pro-collagen	120150
Cole-Carpenter dysplasia	SP	112240	+				
Bruck dysplasia	AR	259450	+				
Singleton-Merton dysplasia	AR						
Osteopenia with radiolucent lesions of the mandible	AD	166260					
Osteoporosis-pseudoglioma dysplasia	AR	259770	–	11q12-q13			
Geroderma osteodysplasticum	AR	231070	–				
Hyper IGE syndrome with osteopenia	AR	147060	–				
Idiopathic juvenile osteroporosis	SP	259750	–				

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
24. Dysplasias with defective mineralization							
Hypophosphatasia-perinatal lethal and infantile forms	AR	241500	+	1p36.1-p34	ALPL	alkaline phosphat-ase	171760
Hypophosphatasia adult form	AD	146300	–	1p36.1-p34			
Hypophosphatemic rickets	XLD	307800	–	Xp22.2-p22.1	PHEX	X-linked hypophos-phatemia protein	171760
Neonatal hyperparathyroidism	AR	239200	+	3q21-q24, 19p13.3	CASR	calcium sensor	601199
Transient neonatal hyperparathyrodism with hypocalciuric hypercalcemia	AD	145980	+	3q21-q24	CASR	calcium sensor	601199
	AD		+	19p13.3			
25. Increased bone density without modification of bone shape							
Osteopetrosis							
Precocious typ4e	AR	259700	+	11q12-13			
Delayed type	AD	166600	–	1p21			
Intermediate type	AR	259710	+				
With renal tubular acidosis	AR	259730	+	8q22	CA2	carbonic anhydrase II	
Axial osteosclerosis							
Osteomeso-pyknosis	AD	166450	–				
With bamboo hair	AR	266500	–				
Pyknodysos tosis	AR	265800	+	1q21	CTSK	cathepsin K	601105
Osteo-sclerosis Stanescu type	AD	122900	+				
Osteopathia striata							
Isolated SP			–				
With cranial sclerosis	AD	166500	–				
Sponastrime dysplasia	AR	271510	+				
Melorheostosis	SP	155950	–				
Osteopoikilosis	AD	166700	–				
Mixed sclerosing bone dysplasia	SP		–				

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
26. Increased bone density with diaphyseal involvement							
Diaphyseal dysplasia	AD	131300	–				
Camurati Engelmann							
Craniodiaphyseal dysplasia	?AR	218300, 122860	+				
Lenz Majewski dysplasia	SP	151050	+				
Endosteal hyperostosis							
van Buchem type	AR	239100	–				
Worth type	AD	144750	–				
Sclerosteosis	AR	269500	–				
With cerebellar hypoplasia	AR	213002	+				
Kenny Caffey dysplasia	AD, AR	127000, 244460	–				
Osteoectasia with hyperphos-phatasia (Juvenile Pagets)	AR	39000	–				
Diaphyseal dysplasia with anemia	AR	231095	–				
Diaphyseal medullary stenosis with bone malignancy (Hardcastle)	AD	112250	–				
27. Increased bone density with metaphyseal involvement							
Pyle dysplasia	AR	265900	–				
Craniometaphyseal dysplasia							
Severe type	AR	218400	+				
Mild type	AD	123000	–	5p15.2-p14.2			
Other types							
Frontometaphyseal dysplasia	XLR	305620	–				
Dysosteosclerosis	AR XLR	224300	–				
Oculodentoosseous dysplasia	AD	257850	+				
	AR	164200	+				
Trichodentoosseous dysplasia	AD	190320	–	17q21			
28. Neonatal severe osteosclerotic dysplasias							
Blomstrand dysplasia	AR	215045	+				
Raine dysplasia	?	259775	+				
Prenatal onset Caffey disease ?	AR	114000	+				

(Continued from previous page)	***Mode of Inheritance***	***OMIM Syndrome***	***Present at Birth***	***Chromosomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
29. Lethal chondrodysplasias with fragmented bones							
Greenberg dysplasia	AR	215140	+				
Dappled diaphyseal dysplasia	AR		+				
Astley-Kendall dysplasia	AR		+				
30. Disorganized development of cartilaginous and fibrous components of the skeleton							
Dysplasia epiphysealis hemimelica	SP	127800	–				
Multiple cartilaginous exostoses	AD	133700	–	8q23-q24.1	EXT1	exostosin-1	
	AD	133701	–	11p12-p11	EXT2	exostosin-2	
	AD	600209	–	19p EXT3			
Enchondromatosis, Ollier	SP	166000	–				
Enchondromatosis with hemangiomata (Maffucci)	SP	166000	–				
Spondyloenchondro-matosis	AR	271550	–				
Spondyloenchondro-matosis with basal ganglia calcification	AR		–				
Dysspondyloenchondro-matosis			–				
Metachondroma-tosis	AD	156250					
Osteoglophonic dysplasia	AD	166250	+				
Genochondromatosis	AD	166000	–				
Carpotarsal osteochondromatosis	AD	127820	–				
Fibrous dysplasia (McCune-Albright and others)	SP mosaic	174800	–	20q13	GNAS1	guanine nucleotide protein, α subunit	139320
Jaffe Campanucci	SP						
Fibrodysplasia ossificans progressiva	AD	135100	+	14q22-q23	BMP4	bone morphogenic protein	4 112262
Cherubism	AD	118400	–				
Cherubism with gingival fibromatosis	AR	135300	–				

(Continued from previous page)	***Mode of Inher-itance***	***OMIM Syn-drome***	***Pre-sent at Birth***	***Chrom-osomal Locus***	***Gene***	***Protein***	***OMIM Gene/ Protein***
31. Osteolyses							
Multicentric predominantly carpal & tarsal in the hand							
Multicentric carpal-tarsal osteolysis with and without nephropathy	AD	166300	–				
Shinohara carpal-tarsal osteolysis			–				
Multicentric predominantly carpal, tarsal and interphalangeal							
Francois syndrome	AR	221800	–				
Winchester syndrome	AR	277950	–				
Torg syndrome	AR	259600	–				
Whyte Hemingway carpal-tarsal phalangeal osteolyses	AD		–				
Predominantly distal phalanges							
Hadju-Cheney syndrome	AD	102500	–				
Giacci familial neurogenic acroosteolysis	AR	201300	–				
Mandibulo acral syndrome	AR	248370	–				
Predominantly involving diaphyses and metaphyses							
Familial expansile osteolysis	AD	174810	–	18q21.1 -q22			
Juvenile hyaline fibromatosis	AR	228600	+				
32. Patella dysplasias							
Nail patella dysplasia	AD	161200	–	9q34.1	NPS1		
Scypho-patellar dysplasia	AD		+				

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